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Pharmacological and morphological effects of *in vitro* transluminal balloon angioplasty in normal and vasospastic canine basilar arteries

by

Patrick Door-Shang Chan ©

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**

in

Experimental Surgery

Department of Surgery

Edmonton, Alberta

Spring 1995

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Pharmacological and morphological effects of in vitro transluminal balloon angioplasty in normal and vasospastic canine basilar arteries**, submitted by Patrick Door-Shang Chan in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.

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This thesis is dedicated to

my life-long companion *Shirley*, and

my parents, *Tai-Ming* and *Hiong-Mooi*

Abstract

Despite growing clinical use of transluminal balloon angioplasty (TBA) to treat cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH), the precise mechanism of action of balloon dilatation on the cerebral arterial wall is unknown. In this experiment the pharmacological and morphological changes in normal (n=10) and vasospastic (n=15) canine basilar arteries following *in vitro* silicone microballoon TBA were examined. For the SAH group in which the double hemorrhage model was employed, vasospasm was confirmed by angiography and the animals were killed on the seventh day following the first SAH. *In vitro* TBA was performed on basilar arteries from normal and SAH dogs immediately after sacrifice and removal of the brain. The procedure was carried out while the arteries were maintained in oxygenated Krebs buffer. In the pharmacological studies, potassium chloride, prostaglandin $F_{2\alpha}$, serotonin, and noradrenaline were used as vasoconstrictors, and bradykinin and calcium ionophore A_{23187} were used to produce an endothelium-dependent dilatation. In both normal and vasospastic groups, the pharmacological responses of dilated segments of basilar arteries were compared to those of nondilated segments of the same arteries.

Vessels from all groups were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM was used to study the intact vessel wall, the smooth muscle cell layer obtained after digestion with hydrochloric acid, and the extracellular matrix obtained after digestion with bleach. Cross-sections of the vessel wall were examined using TEM.

The most striking finding was that immediately after *in vitro* TBA of both normal and vasospastic canine basilar arteries, there was significant reduction ($p<0.05$) of responses to both vasoconstrictors and vasorelaxants. SEM and TEM revealed that both normal and vasospastic vessels dilated with TBA showed flattening and patchy denudation of the endothelium, straightening and occasional rupturing of the internal elastic lamina. In addition, vasospastic vessels dilated with TBA showed decreased surface rippling and mild stretching and straightening of smooth muscle cells, and mild thinning of the tunica media. There was no gross vascular disruption nor an obvious change in the extracellular matrix of the vessel walls of both normal and vasospastic arteries following TBA.

These results suggest that functional impairment of vasoreactivity in the vessel wall as a result of mechanical stretching of the smooth muscle layer plays a more important role than structural alteration, at least in the immediate dilatation produced in vasospastic arteries by TBA.

Key Words - **subarachnoid hemorrhage . canine model . cerebral vasospasm . transluminal balloon angioplasty . mechanism of action . treatment**

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LIST OF ABBREVIATIONS

A23187- calcium ionophore

ADV- adventitia

ADV- adventitia

ANF- atrial naturitic factor

ANOVA- one way analysis of variance

BBB- blood-brain barrier

BK- bradykinin

[Ca2+]- calcium concentration

$CaCl_2$- calcium chloride

CBF- cerebral blood flow

CGRP- calcitonin gene related peptide

CM kinase II- calmodulin kinase II

cNOS- constitutive nitric oxide synthase

CSF- cerebrospinal fluid

DAG- diacyl glycerol

DID- delayed ischemic deficit

ECE- endothelin converting enzyme

ECM- extracellular matrix

ECM-extracellular matrix

EDHF- endothelium derived hyperpolarizing factor

EDRF- endothelium derived relaxing factor

EDT- endothelium

EDT- endothelium

ET- endothelin

ET-1- endothelin-1

ET-2- endothelin-2

ET-3- endothelin-3

eNOS- endothelial nitric oxide synthase

FPCL- fibroblast populated collagen lattice

h- hour(s)

HCl- hydrochloric acid

HHH- hypertensive hypervolemic hemodilutional

HOR- horizontal

5-HT- serotonin

IEL- internal elastic lamina

iNOS- inducible nitric oxide synthase

IP_3- inositol 1,4,5-trisphosphate

KCl- potassium chloride

KH_2PO_4- potassium dihydrogen phosphate

L-NAME- N^G-nitro-L-arginine-methyl-ester

L-NMMA- N^G-nitro-monomethyl-L-arginine

L-NNA- N^G-nitro-L-arginine

LGT- longitudinal

MfPLC- myofibroblast populated collagen lattice

$MgSO_4$- magnesium sulphate

min- minute(s)

mRNA- messenger ribonucleic acid

MLC_{20}- 20 kilodalton myosin light chain

MLCK- myosin light chain kinase

MLCP- myosin light chain dephosphatase

NA- noradrenaline

NaCl- sodium chloride

$NaHCO_3$- sodium bicarbonate

nNOS- neuronal nitric oxide synthase

NO- nitric oxide

NOS- nitric oxide synthase

PC- phosphatidyl choline

PGF2α- prostaglandin F2α

PI- phosphotidyl inositol
PIP2- phosphatidylinositol 4,5-bisphosphate
PKC- protein kinase C
PTA- percutaneous transluminal angioplasty
RP- rippling
RP- rippling
rt-PA- recombinant tissue plasminogen activator
SAH- subarachnoid hemorrhage
SB- smooth border
SB- smooth border
SEM- scanning electron microscopy
SMC- smooth muscle cell
SMC- smooth muscle cells
SR- sarcoplasmic reticulum
TBA- transluminal balloon angioplasty
TEM- transmission electron microscopy
VSR- vasoreactivity

Chapter One: Introduction

Overview

In 1951, Ecker and Riemenschneider (63) reported angiographic vasospasm following aneurysmal subarachnoid hemorrhage (SAH) for the first time. In animal models, SAH often induces an immediate but transient narrowing of cerebral arteries. Brief arterial narrowing of less than 30 minutes is often observed after mechanical irritation of cerebral arteries in animals (17) and is frequently observed during craniotomy in human. However, "early vasospasm" has not been unequivocally documented in humans after aneurysmal SAH. Grote and Hassler (107) did not notice vasospasm in three patients whose aneurysms bled during angiography. Weir et al (94) did not find angiographic vasospasm in SAH patients within 24 hours of ictus. Therefore, the occurrence of early vasospasm in man is questionable, and even if it does exist, its clinical importance seems inconsequential.

"Late" or "chronic" vasospasm which is usually evident angiographically in the second week of SAH (358), is important clinically. Chronic vasospasm affects primarily large conducting arteries which course through the subarachnoid cisterns on the ventral aspect of the brain. These are the arteries upon which saccular aneurysms arise and consequently those which are encased with thick blood clot after aneurysmal bleeding. The significance of this subarachnoid blood clot in the pathogenesis of chronic vasospasm is well documented (120,236). The most prevalent theory remains that the clot, during its gradual dissolution in the subarachnoid space, liberates one or more potent "spasmogens" which mediate sustained cerebrovascular constriction.

Cerebral vasospasm is a delayed-onset, prolonged and pathological cerebral arterial narrowing following aneurysmal SAH, sometimes associated with ischemic neurological deficits (358). The diagnosis of symptomatic cerebral vasospasm should be reserved for patients with delayed-onset focal and/or global neurological deterioration, in which arterial narrowing is confirmed by imaging studies, and other causes of neurological worsening are ruled out. In patients who are in poor neurological condition initially and who do not worsen but fail to improve because of developing vasospasm, the diagnosis of cerebral vasospasm can be difficult. The majority of patients with aneurysmal SAH develop some degree of angiographic cerebral vasospasm, but less than one third of these patients develop symptomatic cerebral ischemia (126,149,150,265).

Cerebral vasospasm used to be described as the "major" cause of morbidity and mortality following aneurysmal SAH. However, proceedings of the 1993 (Fifth) International Symposium on Cerebral Vasospasm held in Edmonton, Alberta, redefined cerebral vasospasm as the largest cause of preventable morbidity and mortality in survivors of aneurysmal SAH (81). In current neurosurgical practice, incidence of patients with poor-outcome secondary to vasospasm is less than 10% (81).

A new and increasingly popular treatment for symptomatic cerebral vasospasm refractory to standard medical treatment is transluminal balloon angioplasty (TBA), which involves mechanical expansion of the spastic artery by means of an inflatable microballoon attached to the tip of an arterial catheter (13,21,24,48,57,64,127,129-131,152,172,176,185,193,195,197,220-223,301,319, 329,382-384,386). The precise effect of TBA on the spastic arterial wall, and the mechanism by which TBA results in prolonged cerebrovascular dilatation, are not yet known.

Prolonged smooth muscle cell contraction may underlie the pathophysiology of cerebral vasospasm (82,83,183,199,215), although an alternative or additional cause of luminal narrowing may be vessel wall contraction arising from proliferation of fibroblasts and increased extracellular fibrous protein matrix -- the so-called "vascular scar" (35,144,296,330,369-371). Thus, the mechanism of the prolonged vasodilatation produced by TBA in vasospastic vessels could arise from damage to the contractile machinery of the smooth muscle cells, from disruption of extracellular connective tissue, or from a combination of these two processes. To investigate these mechanisms further, pharmacological and morphological changes in vasospastic and normal canine basilar arteries following *in vitro* TBA were examined in this study.

Microscopic structure of normal cerebral arteries

To better understand morphological changes in cerebral vessels caused by vasospasm secondary to aneurysmal SAH, and balloon angioplasty, the fine ultrastructure of normal cerebral vessels will be reviewed.

Cerebral vessels can be divided into five main groups, namely artery, arteriole, vein, venule, and capillary. Cerebral vasospasm affects mainly large arteries.

Dahl (50) examined the basilar artery (representing a large cerebral artery), and peripheral branches of the middle cerebral artery (representing small cerebral arteries and arterioles) in the rhesus monkey, rats and hens. All of these cerebral vessels have similar structure and are composed of the usual three coaxial coats: the tunica intima, tunica media, and tunica adventitia. The arterial lumen is lined by a single layer of endothelial cells without fenestrations. In recent years cerebral endothelial cells have been shown to have actin- and myosin-like filaments in the cytoplasm (173,174,241). These myofilaments may enable endothelial cells to contract, potentially contributing to arterial lumen narrowing in vasospasm. One important feature of the cerebral endothelium is the junction between endothelial cells. Tight junctions appear elsewhere in the circulation, but part of the blood-brain-barrier in the central nervous system are tight junctions (uninterrupted) which form an effective obstacle to vascular diffusion (261). A second unique feature of cerebral endothelium is the paucity of pinocytotic vesicles (261). These endothelial cells have comparatively high number of mitochondria, which reflect their high metabolic and thus functional activities (237).

The internal elastic lamina (IEL) in the subendothelial space appears to be made up of a homogeneous matrix, or ground substance, in which the elastic tissue is deposited (237). This ground substance is in close apposition to cell membranes of the overlapping endothelium without any intervening gap. The elastic tissue is concentrated into a single band and borders on the tunica media.

The tunica media is built up of several layers of smooth muscle cells (50,66). The smooth muscle cells are aligned circumferentially except around major bifurcations, where the regular pattern is replaced by multilayer and multidirectional smooth muscle cells. According to Walmsley et al. (350), in human cerebral arteries the tunica media makes up 52% of the total arterial wall thickness while the tunica adventitia and tunica intima account for 31% and 17%, respectively. Smooth muscle cells comprise 72% of the total mass of the tunica media. Adjacent smooth muscle cells in the medial layer are often separated by a single shared basement membrane (66). Since basement membranes follow the contours of their associated cells, they are free to bifurcate or branch complexly wherever intercellular planes diverge. Thus, the entire skeletal framework of the inner tunica of a small cerebral arteriole consists of a continuum of basement membrane surrounding all smooth muscle cells and providing a bed for the endothelium on the luminal side and for the adventitia on the outside. This skeleton serves to link cells one to another and to make the whole vessel wall function as an integrated unit. In contrast to smooth muscle cells that run circumferentially, it is believed that collagen fibers of the adventitia and media and elastin fibers of the intima run parallel to the axis of the artery and perpendicular to the smooth muscle cells (50,66).

In adults, major cerebral arteries have a much thinner wall than those of peripheral arteries of the same caliber, which may partly explain the predisposition of intracranial vessels to development of aneurysms. A possible reason for the thin medial layer is that, for intracranial arteries, the arterial pressure pulse is dampened by the surrounding cerebrospinal fluid within the closed skull (123). As vessels diminish in size, the muscular coat is reduced, until finally, in the small pial arterioles, it consists of only a single layer of muscle cells.

Smooth muscle cells in the wall of muscular arteries contain small areas of conspicuous dense material that lie underneath the cell surface. These structures have been observed previously in smooth muscle of other organ systems and are believed to be attachment devices, anchoring the system of myofilaments to the cell surface at fairly defined points (66). This system of "attachment devices" is particularly prominent and well developed in muscular arteries (66). The tunica adventitia is built up by bundles of collagen which for the most part run either longitudinally or circumferentially. The outer border of the vessel wall has a sharply defined boundary, made up of thin cellular processes which originate from spindle-shaped elongated cells possessing the fine structural features of fibrocytes (50). Where the vessels enter the cerebral cortex there seems to be a membranous junction between this outer lining of the vessel wall and the cytoplasmic processes of the pial arachnoid cells (50).

Extracerebral cranial arteries are furnished with abundant innervation (50,66). The larger vessels are accompanied by periadventitial nerves separated from the outer coat of the vessels. From these nerves, segmental branches enter the vessel wall at regular intervals. When segmental nerves pierce the outer boundary of the adventitial coat (50), they lose their perineurium and become adventitial nerves which arborize within the adventitial coat. In the larger arteries, both myelinated and unmyelinated fibers are seen, whereas only unmyelinated fibers are observed in the arterioles. Without exception, nerve fibers are found only in the adventitial coat, and are never observed within the media (50). Membranous neuromuscular junctions or contacts are not observed (50).

Extracellular "matrix" or connective tissue of vessel wall consists of highly organized substances including collagens, elastins, glycosaminoglycans, proteoglycans, and noncollagenous glycoproteins. This extracellular structure is important for a variety of cellular functions, such as cell growth and development, cell migration and response to injury (188,200). All collagen types (I, II, IV, V, VI, and VIII) present in systemic arteries can also be found in cerebral arteries (188,200,202). Types I and III are fibril-forming interstitial collagens that comprise 80-90% of total arterial collagen, and they can be found in the tunica media and adventitia, and frequently in the tunica intima (200). Type IV collagen is located in basement membrane (26), which also contains laminin, heparan sulfate proteoglycan and entactin. Locations and functions of the remaining three types of collagen (V, VI, and VIII) are unclear at present.

Electron microscopy has demonstrated important ultrastructural differences between cerebral arteries and extracranial arteries (50,66,123). Compared to extracranial arteries which have both external and internal elastic laminae, cerebral arteries have only an internal elastic lamina (50). Different also from extracranial vessels which rely on adventitia for their structural strength and durability, cerebral arteries derive most of their strength from the internal elastic lamina and tunica media. In addition, the collagen structure of cerebral arteries may differ from that of systemic muscular arteries of similar size.

In other organs in the body, arteries receive nutrient supply from vasa vasorum. However, only proximal large cerebral arteries have similar structures. It is unclear in humans how distal cerebral arteries are nourished, although in animals a "rete vasorum" may permit circulation of nourishing cerebrospinal fluid (CSF) through the outer arterial wall. The so-called "rete vasorum" are endothelium-lined intraadventitial channels connected to the subarachnoid space via stomata. The large conducting vessels coursing through subarachnoid cisterns are suspended from brain by many arachnoidal films and trabeculae, and more distal penetrating intracerebral arterioles are surrounded by CSF-filled spaces (Virchow-Robin spaces). This space does not extend to capillary bed, however, where the blood-brain barrier (BBB) exists.

Mechanism of smooth muscle contraction

a) Calcium homeostasis

The contractile state of smooth muscle cells is regulated primarily by free cytosolic (sarcoplasmic) calcium concentration ([Ca^{2+}]). The sarcoplasmic free [Ca^{2+}] in resting smooth muscle cell is in the range of 120-270 nM (52). Upon stimulation, the free [Ca^{2+}] increases transiently, but only to 500-1000 nM (361). The sources of activating calcium are the extracellular milieu, lumen of the sarcoplasmic reticulum (SR), and intracellular calcium store (306). The calcium concentration in these spaces is in the millimolar range. Sarcoplasmic free [Ca^{2+}] is regulated by movement of calcium across the sarcolemmal and SR membranes.

There are two major mechanisms for entry of extracellular calcium in response to appropriate stimuli: voltage-gated and receptor-operated calcium channels (134) (Figure 1). Membrane depolarization or ligand occupancy of membrane receptors opens the calcium channels and allow extracellular calcium to diffuse down the concentration gradient into the sarcoplasm. It should be pointed out that these voltage-dependent calcium channels do not appear to play any significant role in the pathogenesis of cerebral vasospsm, since antagonists of voltage-dependent calcium channels do not prevent contractile response of isolated cerebral arteries to oxyhemoglobin nor reverse established cerebral vasospasm (229,230,357).

The inositol 1,4,5-trisphosphate (IP_3) receptors associated with calreticulin (307,353) on the membranes of SR are also calcium channels. Activation of these receptors allows calcium flow from lumen of SR into sarcoplasm (30). This is followed by entry of extracellular calcium through receptor or second-messenger operated calcium channels (264). The initial rise in intracellular calcium also activates ryanodine receptors on SR to mediate calcium-induced calcium release from the intraluminal calcium-binding proteins (calsequestrin and calreticulin) in SR (139).

Ligands such as prostaglandin F2α, serotonin, norepinephrine and others, act on specific receptors on the sarcolemmal membrane that are coupled via GTP-binding protein to the enzyme phosphoinositide-specific phospholipase C (356). Binding of ligands to the receptors activates phospholipase C to hydrolyze membrane-bound phosphatidylinositol 4,5-bisphosphate, generating two second messengers: lipid soluble 1,2-diacylglycerol (DAG) and water-soluble IP_3 (15). Recently, it is known that agonist-stimulated DAG accumulation is biphasic. Hydrolysis of PIP_2 produces only a transient rise in DAG, followed by a more sustained accumulation of DAG from phosphatidylcholine (PC) degradation by phospholipase C, or concerted actions of phospholipase D and phosphatidate phosphohydrolase (175). DAG activates sarcolemmal membrane-associated protein kinase C (PKC), and IP_3 diffuses across sarcoplasm to interact with IP_3 receptors on the SR membranes (30). To maintain calcium homeostasis at the resting state, and to restore the resting-state calcium concentration after contraction, extrusion of calcium from sarcoplasm has to occur. Sodium/calcium exchangers in the sarcolemma, and calcium pumps in the SR membrane and sarcolemma are the main routes for calcium extrusion (29). Calcium pumps in the sarcolemma and the SR membrane are calcium-transporting ATPase that pump calcium ions out of sarcoplasm into the extracellular environment and the lumen of SR, respectively, at the expense of ATP hydrolysis. Each sodium/calcium exchanger allows three sodium ions to enter the cell in exchange for a single calcium ion. It is coupled to a sarcolemmal sodium/potassium transporting ATPase which pumps sodium that has entered the cell in exchange for calcium, out of the cell in exchange for potassium to maintain the transarcolemmal sodium gradient.

b) The contractile proteins

Organization of contractile filaments in smooth muscle cells is not as well understood as in striated (skeletal and cardiac) muscles. Three types of filaments have been demonstrated in smooth muscle cells: a) thin filaments, composed mainly of actin monomers in double helical strands, and associated with tropomyosin arranged along the length of actin filaments; b) intermediate filaments, composed of desmin or vimentin; and c) thick filaments, composed of aggregated myosin molecules. Intermediate filaments form a skeletal network connecting force-generating units in the cell via the dense bodies and distribute tension throughout the cell. Analogous to their striated muscle counterparts, the thick and thin filaments generate contraction via cross-bridge cycling, in accordance with the sliding filament model of muscle contraction. Contraction occurs by a relative sliding of actin and myosin filaments, without actual shortening of the filaments (135). Sarcomere-like structures have been identified in smooth muscle cells. A sarcomere is the basic contractile unit of striated muscle demarcated by the Z-lines. In smooth muscle cells, actin filaments are attached to dense bodies, analogous to Z-lines of striated muscles. While one end of an actin filament is attached to either a cytoplasmic or a membrane-associated dense body, the other end the the actin filament is not bound but interdigitates with myosin filaments. In turn, the opposite ends of these myosin filaments overlap the free ends of another set of actin filaments, the opposite ends of which are associated with another dense body. Upon contraction of individual sarcomeres, locations on the sarcolemma of associated dense bodies will be pulled toward the center of the cell.

Two isoforms of actin filament exist in smooth muscle, the smooth α and smooth γ (339). The α isoform predominates in vascular smooth muscles and the γ form in enteric smooth muscle. Like striated muscle, tropomyosin is present in smooth muscle and is located in the grooves between the two strands of the actin double helix. In striated muscle, tropomyosin in conjuction with troponin plays an important role in regulating contraction. However, lack of troponin in smooth muscle makes the role of tropomyosin less obvious in these cells.

Smooth muscle myosin is structurally similar to its striated muscle counterpart. Each molecule is a hexamer with two heavy chains and two pairs of light chains (203). Each myosin molecule consists of a long, rodlike tail and two heads. Each globular head has an actin-binding site and a site of ATP hydrolysis. Thus, myosin is a magnesium-ATPase.

c) Myosin phosphorylation

Following binding of appropriate agonists to sarcolemmal receptors, the cytosolic free calcium concentration rises. Intracellular free calcium ions bind calmodulin in 4:1 ratio (352). Binding of calcium to calmodulin induces conformational change in the latter, exposing one or more active sites on calmodulin that in turn bind to and activate myosin light chain kinase (MLCK). Activated MLCK catalyzes phosphorylation of myosin at S19 in each of the two

20-kDa light chains, triggering cycling of myosin cross-bridges along the actin filament and smooth muscle contraction (122).

Relaxation of smooth muscle occurs more or less through the reversal of the sequence of events mentioned above (352). The cytosolic free calcium concentration returns to the resting level through the mechanisms operating on the channels located in sarcolemma and the SR membrane mentioned earlier. Dissociation of calcium and calmodulin from MLCK follows the lowering of free cytosolic calcium concentration, and the inactive form of MLCK is generated. Phosphorylation of myosin ceases, and the myosin that had been phosphorylated during the contractile state is dephosphorylated by myosin light chain dephosphatase (244). Dephosphorylated myosin detaches from actin, and smooth muscle relaxes.

d) Secondary calcium-dependent control mechanisms

Although myosin phosphorylation-dephosphorylation is important in generating smooth muscle contraction, other secondary calcium-dependent mechanisms exist that can modulate actin-myosin interaction independent of myosin phosphorylation mentioned earlier (145). Studies have indicated that, in the so-called "latch state", prolonged tension can be maintained in smooth muscle at a low level of myosin phosphorylation, but still significantly above the level during full muscle relaxation. Dephosphorylation of an attached cross-bridge gives rise to a long-lasting, noncycling latch-bridge. Latch bridge formation is a form of energy conservation during prolonged tension generation because significantly less amount of ATP is hydrolyzed during force maintenance than during force development. This maintenance of prolonged tonic contraction is achieved by noncycling or slowly cycling cross-bridges (111). Latch bridges are calcium-dependent and relaxation in smooth muscle occurs when the cytosolic calcium concentration returns to the resting level (2). On the contrary, Rasmussen and coworkers (260) suggested that the tonic phase of contraction of traditional vasoconstrictors is maintained not exclusively by formation of a latch bridge between actin and myosin, but rather by a reorganization of cytoskeletal elements induced by protein kinase C-dependent phosphorylations (55).

In vertebrate smooth muscle, there exits a thin filament-linked regulatory mechanism in addition to the thick filament-linked myosin phosphorylation system in the regulation of actin-myosin interaction. The regulatory proteins involved are called caldesmon and calponin.

Caldesmon means "calmodulin-binding" although it also binds actin in smooth muscle and nonmuscle cells (303). *In vitro* interaction of caldesmon with calmodulin is calcium-dependent but the interaction with actin, tropomyosin and myosin is calcium-independent (352). There are two forms of actin-containing domains in smooth muscle, the actomyosin domain and the actin-intermediate filament domain. Caldesmon is exclusively located in the actomyosin domain, stretching out along the actin double helix in register with tropomyosin. Electron microscopic studies indicated that caldesmon binds to actin filament rather than tropomyosin (211). The function of caldesmon is to inhibit activity of actin-activated myosin magnesium-ATPase. Phosphorylation of caldesmon by calcium-calmodulin-dependent protein kinase II (CaM kinase II) results in loss of its affinity for myosin and its inhibition of the actin-activated myosin magnesium-ATPase, allowing cross-bridge cycling to occur (224). Caldesmon can cross-link actin to myosin. This cross-linking is thought to represent latch-bridge formation, and may organize the contractile elements into a three-dimensional network capable of tension generation in the presence of appropriate stimuli (352).

Calponin is an actin- and calmodulin-binding protein found exclusively in smooth muscle (363-365). It binds to calmodulin in a calcium-dependent manner, and to actin in a calcium-independent manner (320). It inhibits actin-activated magnesium-ATPase activity of smooth and skeletal muscle myosins through its interaction with actin; myosin phosphorylation is unaffected. When phosphorylated by CaM kinase II or protein kinase C (PKC) in the presence of elevated free cytosolic calcium concentration, it dissociates from actin and loses its ability to inhibit the actomyosin ATPase (364). The dissociation of calponin from actin allows maximal phosphorylation by MLCK and cross-bridge formation, thus maximal tension development. The level of calponin phosphorylation correlates with the velocity of muscle shortening. Fine-tuning of the velocity of muscle shortening can be achieved by altering the relative amounts of phosphorylated and dephosphorylated calponin and myosin.

Finally, it should be mentioned that calcium-independent and phospholipid-diacylglycerol dependent isoenzymes of sarcolemmal PKC have been increasingly recognized to play an important role in regulation of contraction and other cellular functions in smooth muscle (145,175,227). Activation of PKC in vascular smooth muscle is known to modulate agonist-stimulated phospholipid turnover, increase generation of contractile force, and regulate cell growth and proliferation (175).

While phasic smooth muscle contraction mediated by the calmodulin system and actin-myosin interaction is well understood, mechanism of tonic contraction, which probably occurs in chronic arterial narrowing after SAH, remains speculative (317,344,346). Rasmussen et al (260) used a coordinate fibrillar domain model of contraction to propose different cellular and molecular events governing the initial and sustained phases of smooth muscle contraction. This model differs from accepted views, particularly the latch bridge hypothesis, in terms of both calcium messenger function and the molecular events underlying this process. According to this new model, the initial phase of response is mediated by a rise in cytosolic calcium concentration and resulting camodulin-dependent activation of both myosin light chain kinase and dissociation of caldesmon from the actin-caldesmon-tropomyosin-myosin fibrillar domain. These events lead to an interaction between actin and the phosphorylated light chains of myosin just as in the most widely accepted model discussed earlier. However, this initial phase is followed by a sustained phase in which a rise in intracellular calcium concentration stimulates the plasma membrane-associated, calcium-sensitive form of PKC that results in phosphorylation of both structural and regulatory (namely calponin) components of the filamin-actin-desmin-fibrillar domain.

Regulation of cerebral vascular tone by endothelium

Endothelial cells play an important role in regulating the vascular tone of cerebral vasculature (76,96). These cells are known to produce a number of relaxing factors, including endothelium-dependent relaxing factor (EDRF) (75), prostacyclin, endothelium-derived hyperpolarizing factor (EDHF), and oxygen-derived free radicals (76). Among these vasorelaxants, the most extensively studied and most important one is EDRF. In extracranial vessels, EDRF has been identified as nitric oxide (NO) or a labile nitroso compound containing NO, such as S-nitrocysteine, produced from the amino acid L-arginine by NO-synthase (NOS) (210,214). In cerebral arterioles, EDRF is thought to be an NO-containing compound rather than NO per se (267). However, the L-arginine-NO pathway has been identified in large cerebral arteries (23,232). The agonists known to activate this pathway include bradykinin, A_{23187}, substance P, acetylcholine, adenosine phosphates, thrombin, neurokinins, neuropeptide K, vasopressin, oxytocin, and histamine (44,73,331).

In cerebral arteries, endothelial cells contain NOS but smooth muscle cells do not (23,232). Two major isoforms of NOS, constitutive and inducible, have been identified. These two forms of NOS are regulated by different mechanisms (56,210). The inducible NOS (iNOS), present in macrophages, has negligible basal activity but increases its activity significantly in 2 to 4 hours following stimulation by lipopolysaccharide and/or gamma interferon (154). The isoform present in neurons (nNOS) and endothelial cells (eNOS) is expressed constitutively (89,210). The inducible NOS is calcium independent for its intracellular activity. On the contrary, the constitutive NOS (cNOS) is activated by calcium, which binds to calmodulin, forming a complex which acts as a cofactor for enzyme function (89,210). When the membrane receptors of endothelial cells are stimulated by agonists, such as bradykinin and substance P, inositol 1,4,5-trisphosphate (IP_3) is generated via the phosphoinositide second-messenger system (285). Inositol 1,4,5-trisphosphate stimulates calcium release from intracellular stores by binding to IP_3 receptors on the endoplasmic reticulum. This rise in cytosolic calcium promotes entry of extracellular calcium. Contrary to agonists acting through receptor-mediated mechanisms, the calcium ionophore A_{23187} promotes direct entry of extracellular calcium (56,159,210,285). The calcium-calmodulin complex facilitates production of NO from L-arginine by NOS (Figure 2). NO diffuses across endothelial cells to nearby smooth muscle cells. NO relaxes blood vessels by binding to iron in the heme at the active site of guanylate cyclase in smooth muscle cells, thereby activating the enzyme to generate cGMP (210). cGMP may elicit muscle relaxation through influences on a sodium-calcium exchange system, by stimulating the phosphorylation of poorly defined substrates by cGMP-dependent protein kinase, via direct action at a cGMP-coupled second messenger system, or as a consequence of cGMP-mediated activation or inhibition of phosphodiesterases (159,210). Nitrovasodilators, such as sodium nitroprusside and nitroglycerin, which are endothelium-independent vasodilators, directly increase formation of cGMP and produce relaxation of cerebral vascular muscle cells (132,354). Methylene blue inhibits soluble guanylate cyclase, reducing the relaxation of cerebral vessels in response to NO and nitroprusside (153,156,354). The formation of NO from L-arginine by NOS can be inhibited by N^G-substituted analogues of L-arginine, such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA), and N^G-nitro-L-arginine methyl-ester (L-NAME) (89,210). These inhibitors produce a dose-dependent constriction in cerebral blood vessels by lowering the basal level of cGMP in the presence of intact endothelium.

The basal release of EDRF from endothelial cells is important in counteracting the constrictor response of cerebral vessels to vasoconstrictors such as norepinephrine, serotonin, and prostaglandins. Basal EDRF exerts more influence in the vascular tone of large cerebral arteries than that of smaller cerebral vessels (74). On the other hand, relaxing factors such as oxygen-derived free radicals appear to dominate over EDRF in regulation of basal vascular tone of cerebral arterioles (76). It has been shown that the response of cerebral arterioles to bradykinin was not mediated by EDRF, and this response could be inhibited by superoxide dismutase, catalase and deferoxamine, suggesting that hydroxyl radicals could be involved in mediating vasorelaxation in cerebral arterioles (166,266). Thus, there appears to be two mechanisms of endothelium-dependent relaxation in cerebral vessels, depending on the size of vessels involved. EDRF mediates endothelium-dependent relaxation in larger cerebral vessels, such as basilar and middle cerebral arteries, while oxygen free radicals mediate the response in pial arterioles.

Endothelium-dependent relaxation is impaired in a number of pathophysiological conditions, such as vasospasm following aneurysmal SAH, cerebral ischemia, stroke, atherosclerosis and chronic hypertension (76). The impairment is related to alteration in the basal release of EDRF and/or production of endothelium-derived vasoconstrictors.

In SAH of animal models and humans, several mechanisms related to impairment in endothelium-dependent relaxation may contribute to vasospasm. First, the activity of soluble guanylate cyclase may be reduced, as shown in a canine model of SAH (155,156). Second, in addition to its direct constrictor response in smooth muscle cells, hemoglobin avidly binds NO and destroys NO by generation of superoxide anions, thus decreasing the availability of NO to smooth muscle cells (184). Third, endothelin levels in basilar arteries and cerebrospinal fluid (CSF) are increased after SAH (168,192,314,373). Endothelin is produced by endothelium and it is an extremely potent and long-lasting polypeptide vasoconstrictor (191). Both thrombin and oxyhemoglobin in thrombus and CSF from SAH patients have been shown to induce endothelin gene expression and increase endothelin production (191,315).

Pathogenesis of cerebral vasospasm

Angiographic vasospasm usually begins within 3 to 5 days of SAH, and peaks both in incidence and severity between the first and second week, and then gradually resolves by the third to fourth week following hemorrhage (358). Infrequently, vasospasm may last longer than 3 weeks.

The mechanism by which luminal narrowing develops following SAH is the subject of two main hypotheses, "the vasoconstriction hypothesis" and "the structural hypothesis". It is still uncertain whether vasospasm is an active muscle-mediated vascular constriction (83,183,185,196,215), or a non-muscle related structural change in the vessel wall resulting in luminal narrowing.

a) *vasoconstriction hypothesis*

Cerebral arteries pharmacologically vasoconstricted *in vitro* demonstrated impressive medial thickening that was indistinguishable from thickening observed in vasospastic arteries seven days after hemorrhage (183). In addition to morphological evidence of smooth muscle contraction in vasospasm, a large body of evidence for biochemical and physiological changes in smooth muscle cells are also present. Over the past two decades, many substances have been implicated as potential mediators for vasospasm. These potential mediators include hemoglobin, oxyhemoglobin, methemoglobin, lipid peroxides, eicosanoids, bilirubin, endothelins, neurohumoral factors and others (82). In 1979, Fox showed that iron compounds could induce cerebral vasospasm. Todate it is generally accepted that hemoglobin is the principal mediator or trigger of a process that results in vasospasm (184). This postulate is based on several lines of evidence. First, oxyhemoglobin level is elevated in CSF of patients with aneurysmal SAH throughout the time course of vasospasm (235). Second, hemoglobin, and particularly oxyhemoglobin, contract isolated smooth muscle cells and arteries of many different animal species *in vitro* and *in vivo* (95,184,198,328,336,359), and this effect seems most marked on cerebral vessels. Third, oxyhemoglobin has been shown to induce morphological changes characteristic of vasospastic cerebral arteries in cultured arterial smooth muscle cells (95) and cerebral arterial walls in an experimental SAH model (184).

Aged erythrocytes and their degradation products are the ingredients of blood clot that produce vasospasm in dogs, cats, and pigs (120,184,236,240). Hemolysis is necessary for vasospasm to develop (184), and *in vitro* experiments indicate that oxyhemoglobin is the vasoactive substance released during hemolysis (184). Methemoglobin is an oxidized form of oxyhemoglobin in which the ferrous ion is substituted with a ferric iron. While oxyhemoglobin is capable of eliciting strong and prolonged smooth muscle contraction, methemoglobin is an ineffective vasoconstrictor (187). This is because ferrous ion in oxyhemoglobin is able to catalyze free radical reaction but ferric ion in methemoglobin fails to do so (116). Despite a large body of evidence for hemoglobin as the initiator in the development of vasospasm, the intracellular mechanism responsible for this process remains unclear. It has been suggested that oxyhemoglobin may activate phospholipase A2 to produce arachidonic acid (98), which can sensitize tissue to calcium. Phosphorylation of myosin light chain can then be maintained in the presence of constant calcium concentration, enabling smooth muscle contraction to occur (103). Oxyhemoglobin can attenuate the effect of EDRF (76). It decreases the basal level of cGMP that mediates the vasorelaxation effect of NO in smooth muscle cells (285). However, the effect of oxyhemoglobin on NO is insufficient to account for cerebral vasospasm since oxyhemoglobin can elicit vasoconstriction even in cerebral arteries denuded of endothelium (156,336). The search for the ultimate spasmogen in subarachnoid blood clot has dwindled and, most of the workers in cerebral vasospasm are concentrating on the intra- and extracellular molecular changes induced by subarachnoid blood and hemoglobin.

Increasing amount of evidence suggests that free radical formation and peroxidation are important in cerebral vasospasm. Oxyhemoglobin can increase production of lipid peroxides that can damage cellular membranes and induce contractile response (164,308). The close temporal association of cerebral arterial narrowing with oxygen free radical formation and lipid peroxidation suggests an important role for these mechanisms (140,279). The degree of vasospasm is correlated with the concentration of lipid peroxides in the cerebrospinal fluid in patients with SAH (10,281,355). In an experimental SAH, Sasaki et al (281,282) noted that the concentrations of lipid peroxides in the cerebrospinal fluid and in the arterial wall were increased substantially after SAH. Intracisternal injection of a lipid hydroperoxide, 15-hydroperoxyarachidonic acid (15-HPAA), mimics chronic vasospasm observed after SAH (282). Furthermore, treatments aiming directly or indirectly at reducing the generation of lipid peroxides facilitate blood flow and reduce vasospasm after SAH (334,342). Taken togather, these data support the hypothesis that lipid peroxidation, triggered by lysis of erythrocytes in the subarachnoid clot, participates in the process of cerebral vasospasm. Autooxidation of oxyhemoglobin produces methemoglobin and superoxide anion radicals (91,206). Superoxide radicals in the presence of water generate hydrogen peroxide, which reacts with the ferrous molecule in hemoglobin and releases hydoxyl free radicals (110,360). These free radicals catalyze lipid peroxidation in the membranes of smooth muscle cells via Haber-Weiss or Fenton reaction (110), which in turn activates phospholipases, including phospholipase C, in the plasma membranes of smooth muscle cells (343,344,346). Phospholipase C triggers a chain of intracellular signallings which lead to elevation of intracellular calcium concentration and ultimately smooth muscle contraction as described before. Lipid peroxidation also triggers calcium influx from extracellular to intracellular compartment (343,344,346). These findings suggest that antiperoxidants and free radical scavengers might be useful therapeutic candidates for ameliorating SAH-induced vasospasm. Indeed, lazaroids, which are 21-aminosteroids and strong scavengers of oxygen free radiacals, have been shown to be effective inhibitors of iron-catalyzed lipid peroxidation in animal models of SAH (90,115). These compounds can also reduce vasospasm in both animal studies and human trials (90,113,146,147,385).

There is evidence that calcium homeostasis is perturbed in smooth muscle cells of vasospastic vessels, although it is still not clear whether calcium entry and sustained elevation of intracellular calcium concentration are necessary for tonic contraction (260,352). Calcium conductance has been shown to increase significantly in cerebral smooth muscle cells exposed to oxyhemoglobin (286). Takayasu et al (323) showed that HA 1077, an intracellular calcium antagonist, effectively antagonized vasospasm in dogs. Studies showed that exposing cultured smooth muscle cells of cerebral arteries to oxyhemoglobin induced a rapid and a continual rise of intracellular calcium concentration over several days (321,346). Vollrath et al (344-346) noted that oxyhemoglobin produced a transient but highly significant rise in the intracellular concentration of inositol triphosphate (IP_3). These studies suggest that elevation of IP_3 and sustained rise of intracellular calcium concentration are important at least in the early development of vasospasm. However, whether these two processes are related remains speculative. Sustained elevation of intracellular calcium level was abolished by neomycin, a phospholipase C inhibitor, denoting that activation of phospholipase C is important in the pathogenesis of vasospasm (346). It is possible that oxyhemoglobin acts through PLC pathway to release IP_3 which increases intracellular calcium concentration as discussed earlier. However, that elevation of IP_3 is only transient implies that other PLC-dependent mechanisms also contribute to sustained contraction seen in SAH-induced vasospasm (346).

Asano and colleagues (9) showed that cerebral arteries with established vasospasm had a marked elevation of DAG, which was not accountable by the breakdown of PIP_2 alone. The source and mechanism for the increase in DAG has not been established. DAG is known to activate PKC, which plays a crucial role in the signal transduction of normal smooth muscle contraction (175,352) and has been shown to be involved in the development of vasospasm after SAH (9). It is believed that the initial rise in intracellular calcium is responsible for the phasic contraction of smooth muscle cells, but activation of PKC pathway may account for the tonic contraction of smooth muscle cells in chronic cerebral vasospasm (170,226,260,313,343,344,346). Activation of PKC may mediate non-specific constriction to a number of agents during the early phase of vasospasm. A secondary role for PKC related to its mitogenic effects may be important in the structural alterations commonly observed during the later phase of SAH. Antagonists of protein kinase C (H7 and staurosporine) could prevent vasospasm in dogs (193). Nishizawa et al (226) measured PKC activity in the membranes and cytosol of vasospastic canine basilar arteries with (γ-^{32}P)-adenosine triphosphate.

Because PKC is translocated to the membane from the cytosol when it is activated, the translocation is an index of the activation. The authors found that membrane PKC activity in the SAH model was significantly increased compared to that in the control group. The percentage of membrane activity to the total was greater but the percentage of cytosol activity was less in the SAH vessels than in the control arteries. The results indicated that PKC in vascular smooth muscle was translocated from cytosol to membrane and was activated after SAH. These investigators later explored the relation between PKC and calmodulin systems in tonic contraction of cerebral vessels. They noted that this tonic phase was dependent on the PKC system, but initiation of contraction by the calmodulin system was necessary for the subsequent PKC-dependent tonic contraction. They also demonstrated that specific calmodulin antagonists could partially but not completely inhibit tonic contraction associated with PKC activation.

Despite much evidence correlating PKC change with development of vasospasm, some studies do not support this correlation. Takanashi et al (321) found that the level of PKC was not elevated in the cultured cerebral smooth muscle cells exposed to hemolysate. Minami et al (205) showed that the function of PKC in experimental SAH model is not the same as that in vessels with pharmacologically-induced tonic contraction. The investigators cautioned the analogy between vasospastic and tonic vessels, and suggested that vasospasm might not represent tonic contraction.

Preliminary studies suggest that other components in the intracellular pathway of normal smooth muscle contraction, such as calmodulin and calponin, are altered after SAH. Doi and his colleagues (58) have shown that in spastic vessels, both calcium binding proteins calmodulin and caldesmon (59), and calponin (58), are functioning in a different manner from their roles in normal arteries. The activity of μ-calpain resulting from the balance of μ-calpain and calpastatin is enhanced continuously in vasospastic vessels; this continuous activation of μ-calpain probably induces proteolytic changes of regulatory and structural proteins in vasospastic vessels (372). In most agonist-mediated vascular smooth muscle cell contraction, intracellular calcium and calmodulin mediate phosphorylation of 20 kDa myosin light chain (MLC_{20}), which promotes contractile force generation through actin-myosin ATPase; however, there is no significant difference between the control and spastic canine basilar arteries in the phosphorylation level of MLC_{20} (119), suggesting that a different mechanism, other than phosphorylation of MLC_{20} might be responsible for chronic vasospasm.

In summary, numerous studies have illustrated that signal transduction and regulatory and contractile proteins in normal smooth muscle contraction can be altered to maintain persistent narrowing of cerebral arteries after SAH. Biochemical disturbance alone, with or without accompanying structural alteration, in smooth muscle cells may explain the chronic spastic state in cerebral vasospasm.

Sustained vasoconstriction may produce alteration in the pharmacological and structural properties of smooth muscle cells. Irrigating exposed feline basilar arteries with either serotonin or calcium gluconate, Yoshioka et al (378) found that vasoconstriction longer than ten hours was both pharmacologically irreversible and associated with myonecrotic changes seen with electron microscopy. Fujii and Fujitsu (95) found that incubation of cultured arterial smooth muscle cells with oxyhemoglobin for 24 hours resulted in irreversible progressive contraction of the cells and ultrastructural changes resembling myonecrosis. It seems possible, therefore, that prolonged constriction in response to oxyhemoglobin and/or its vasoactive byproducts such as lipid peroxides or prostaglandins induces a temporarily irreversible state associated with ultrastructural myonecrotic change. Although this pathophysiological process has not yet been fully elucidated, it is known that in the majority of cases vasospasm spontaneously resolves within several weeks of its onset (82,83,183,184,358). However, there are rare instances in which cerebral vasospasm leads to persistent arterial stenosis (246). As originally suggested by Conway and McDonald (246) it is likely that this is due to prominent intimal thickening, which may reflect a nonspecific pathological response related to the severity of the vessel wall injury. Some degree of intimal injury associated with ultrastructural alterations is probably inevitable in cerebral vasospasm, due to either hypoxia related to prolonged vasoconstriction or the release of toxic substances from the decaying periarterial clot.

Discussion of the pathogenesis of cerebral vasospasm is incomplete without the mention of endothelin, which has been strongly implicated in maintaining chronic narrowing in cerebral arteries after SAH (8,374,375). Endothelin has both potent and long-lasting vasoconstrictor effects on cerebral vascular smooth muscle *in vivo* and *in vitro* (160,274), which are greater than those of any other vasoconstrictor (274). This fact makes endothelin an attractive potential mediator of chronic arterial narrowing in cerebral vasospasm.

Three distinct but structurally and pharmacologically related members of the endothelin family have been identified, namely endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). Although the relation of ET-1 to cerebral vasospasm has been widely studied, the role of the other two isoforms of endothelins in this disease process is obscure. The only isoform of endothelin produced by endothelial cells is ET-1 (374,375). Release of ET-1 from endothelial cells can be stimulated by oxyhemoglobin, thrombin, arginine vasospressin, angiiotensin II, or transforming growth factor-β (284,375). Agents capable of stimulating the synthesis of ET-1 in cultured human vascular smooth muscle cells include angiotensin II, arginine vasopressin, transforming growth factor-β, platelet-derived growth factor AA, and epidermal growth factor (262). Most of these ET-1-inducing substances are present in the subarachnoid blood clot following aneurysm rupture.

The intracellular mechanism of ET-1 production is well established (191,284). Biosynthesis of endothelin is regulated at the messenger ribonucleic acid (mRNA) transcription level (142,374). Preproendothelin is converted to big ET-1 by a putative endothelin-converting enzyme (ECE) (191,284). Smooth muscle contraction induced by ET-1 is resistant to the following antagonists: α-adrenergic, H_1-histaminergic, serotonergic, cyclo-oxygenase, and lipoxygenase (374). This suggests that endothelins act on membrane receptors different from those of other vasoconstrictors on vascular smooth muscle cells. Two different ET receptor subtypes have been identified and cloned: ET_A and ET_B (5,277). There are evidence for other subtypes (304). The ET_A receptor has a greater affinity for ET-1 and ET-2, whereas ET_B receptor binds all three endothelins with equal affinity (5,277). The ET_A receptor mediates a major part of the vasoconstriction induced by ET-1(138). The ET_B subtype mediates endothelium-dependent vasodilation (322), but its stimulation by selective agonists can also induce vasoconstriction (38). ET-1 may be produced by cerebral arterial endothelial cells (377). In addition to acting directly on ET_A receptors of smooth muscle cells to produce vessel wall contraction, this polypeptide may also sensitize blood vessels to other constricting substances (40,101,376). This observation can explain the prolonged and abnormal smooth muscle constriction in cerebral vasospasm.

ET-1 induced vasoconstriction is dependent on availability of extracellular calcium (374), and it is significantly reduced in the presence of calcium channel blocker, nicardipine (374). Several lines of experimental evidence suggest that ET-1 may act through phospholipase C pathway leading to breakdown of intramembrane phosphoinositide and subsequent intracellular calcium mobilization and protein kinase C activation to cause smooth muscle contraction (7,106,162,189,213,262,263,311,312). Griendling et al showed that endothelin stimulates DAG accumulation and activates PKC in cultured vascular smooth muscle cells (106). These intracellular signallings may contribute to the physiologic events stimulated by endothelin in intact blood vessels, such as slow tonic contraction and calcium influx (106). Studies have shown *in vitro* and *in vivo* vasoconstrictor effect of ET-1 in cerebral arteries. Isolated canine basilar arteries are reported to contract in a dose-dependent manner in response to ET-1 (8,136). Intracisternal injection of ET-1 can induce sustained narrowing of canine basilar arteries for 24 hours and even up to 3 days (8). However, intra-arterial administration of ET-1 has no appreciable effect on the caliber of normal canine basilar artery (204). It should be noted that ET-1 causes vasoconstriction of cerebral arteries only from the adventitial side (204). Perhaps blood brain barrier prevents intraluminal or circulating ET-1 from reaching vascular smooth muscle cells.

Of importance to researchers in cerebral vasospasm is that oxyhemoglobin can directly stimulate cultured endothelial cells and cultured smooth muscle cells to produce endothelin (233). Kasuya and colleagues (151) suggested that oxyhemoglobin-induced ET-1 production in endothelial cells is regulated by PKC, and in smooth muscle cells by both PKC and cAMP-dependent pathway. Oxyhemoglobin can also potentiate stimulation of immunoreactive endothelin production by platelets, which may be one of the endogenous regulators of endothelin biosynthesis (233). High concentration of oxyhemoglobin can bind NO and decrease cGMP level, thus increasing production of ET-1 (20). Endothelin formation has been shown to be affected by endothelium-derived NO through a cGMP-dependent mechanism (20). However, a contrary study showed that despite marked increases observed in endothelial release of endothelium-derived NO, endothelial levels of cGMP remained mostly unaffected (233). ET-1 produced by endothelial cells in cerebral arteries can gain access to the membrane receptors of adjacent smooth muscle cells to produce vessel wall contraction (377). However, it is still controversial if elevation of ET-1 level in plasma or CSF plays any role in the pathogenesis of cerebral vasospasm. Several studies have demonstrated increased immunoreactivity level of ET-1 in the plasma and CSF of patients or animals with SAH (194,314,316), but other studies did not (45,117,290). It should be emphasized that ET-1 level in the plasma or CSF after SAH does not necessarily reflect local availability or concentration of this peptide in the vessel wall. Yamaura and coworkers (373) showed that the immunoreactive ET-1 level in the basilar arterial wall was significantly elevated on day 2 of SAH but not significantly changed on day 7 of SAH when angiographic vasospasm was evident. Cultured endothelial cells are known to secrete twice as much ET-1 towards adjacent smooth muscle (the basolateral side) than towards the luminal side (348,377), suggesting that ET-1 is a paracrine factor and not a hormone.

Actinomycin D, an inhibitor of ribonucleic acid synthesis, given for 5 days after SAH, completely suppressed the development of vasospasm (289). Recently, ECE inhibitors or ET_A receptor antagonists have been shown to prevent or reduce cerebral vasospasm. Phosphoramidon, a nonspecific metalloprotease inhibitor, is known to inhibit the conversion of big ET-1 to ET-1 by ECE (141,283). Because the vasoconstrictive potency of big ET-1 is considerably less than that of ET-1, the inhibition of ECE should ameliorate the biological effects of ET-1. Indeed, intracisternal administration of phosphoramidon has been shown to decrease cerebral arterial narrowing induced by intracisternal big ET-1 (293). In addition, intracisternal pretreatment of phosphoramidon prevented cerebral vasospasm following SAH in a canine model (194). However, other studies did not elicit any preventive effect of this inhibitor in the development of cerebral vasospasm after SAH (45,46,290).The ET-1 receptor antagonists used in experimental models of SAH include synthetic peptides named BQ123, BQ485, and FR139317 (37,45,143,225). BQ123 is a specific ET_A receptor antagonist that does not cross blood brain barrier. It prevents early cerebral arterial narrowing following SAH after intracisternal but not intravenous injection (37). Contrary studies showed that daily intracisternal administration of BQ123 inhibited contraction induced by ET-1 but did not prevent experimentally induced cerebral vasospasm (117). Recently, Clozel et al (37) showed that compound Ro 46-2005, a modified pyrimidinyl sulphonamide, completely inhibited the specific binding of ET-1 to human vascular smooth muscle cells (ET_A receptor) and rat aortic endothelial cells (ET_B receptor). Ro 46-2005 is the first synthetic orally active nonpeptide antagonist of endothelin receptors (37).

In summary, the role of endothelin in the pathogenesis of cerebral vasospasm is controversial. Further studies are required to clarify this controversy.

b) structural hypothesis

The structural hypothesis of vasospasm proposes that chronic vasospasm represents an injury response of the vessel independent of vasoconstriction, and involves necrosis, edema, leukocytic infiltration, cellular proliferation, and fibrosis within the vessel wall. According to this theory, luminal narrowing is due to vessel wall thickening and fibrosis (14,144,296,297,299,369-371). Proponents have sought to deemphasize the role of smooth muscle spasm per se in the genesis of delayed arterial narrowing. They argue that the acutely constricted (early vasospasm) smooth muscle cells become "locked" into their shortened configuration by a deposition and/or rearrangement of the extracellular collagen matrix of the vessel wall, mediated by myofibroblasts that proliferate in cerebral arterial wall exposed to subarachnoid blood and/or in chronic spasm.This theory is based on a number of important observations. Clinical vasospasm has thus far proven resistant to pharmacological vasodilatation, suggesting that the narrowing is not a physiological vascular constriction. Furthermore, spastic vessels obtained from primates after experimental SAH have been shown to have relatively rigid walls compared to control animals (16,215), and markedly diminished *in vitro* contractile responses to agonists such as norepinephrine, 5-hydroxytryptamine, and potassium chloride (16,19,74,216,332). Clower et al (35) reported decreased vessel elasticity 6 days after SAH in a monkey model. It has been shown that the modest mean increase in wall thickness could not account for increased wall stiffness in vasospastic vessels. Kim et al (158) showed that the rigidity of vessel wall was increased in the presence of total inhibition of myogenic tone, emphasizing the importance of non-contractile component of the vessel wall in SAH. Morphological changes have been described in human autopsy cases of cerebral vasospasm (43,246,297,299) and experimental SAH (35,80,177,246,325,327), the striking features being cellular proliferation and increased extracellular matrix in the subintimal layer and the tunica media (35,246,297,299), and myonecrosis.

Yamamoto and colleagues (144,296,330,369,371) used myofibroblast populated collagen lattice (MfPCL) system, a modified fibroblast populated collagen lattice (FPCL) system, to study the pathogenesis and morphological changes related to vasospasm (369). The FPCL system is a three-dimentional *in vitro* model that simulates wound healing, scar contracture, and fibrosis (68,309). It is created by mixing cell suspension and collagen extraction togather and allowing the mixture to polymerize, thus trapping the cells in the resulting lattice matrix. Yamamoto et al (369) found that, upon suspension in a three-dimentional collagen matrix, the myofibroblasts obtained from cerebral arteries taken at autopsies from patients who died of cerebral vasospasm would extend filopodia into the matrix within 24 hours. The cells rearranged the structural network of collagen by pulling collagen binding sites along their surfaces, compressing the collagen matrix. Reorganization of collagen fibrils and MfPCL compaction occur in the absence of cellular shortening observed in the constriction of smooth muscle cells. Accordingly, the constriction in vasospastic vessels is considered the results of the action of myofibroblasts on collagen, and perhaps on other components of the extracellular matrix (369). Bloody CSF from patients with symptomatic vasospasm accelerated MfPCL contraction (369). These authors also found that, when stimulated by CSF from patients with symptomatic vasospasm, myofibroblasts could compact collagen lattice at a rate equivalent to that of human dermal fibroblasts (369). MfPCL compaction was inhibited by verapamil or heparin but not by nimodipine or nicardipine (371). This observation suggests that proliferative cells appearing in vasospastic arteries have characteristics different from those of differentiated smooth muscle cells in the medial layer and could generate a force rearranging the proliferated collagen matrix present after SAH. Rearrangement of extracellular matrix can contribute to, or be responsible for, sustained vasoconstriction. It might explain the difficulty in treating chronic vasospasm with pharmacological dilators directed toward constricted smooth muscle, and the long-lasting mechanical dilatation in vasospastic vessels with balloon angioplasty.

The results from the studies in MfPCL system have limitations. The cell lines used to populate the lattice system were derived from the media of cerebral arteries taken at autopsy from a few patients with cerebral vasospasm. The definite identity of these cells is still debatable, although the authors noted in these cells a limited number of ultrastructural and immunohistochemical characteristics consistent with myofibroblasts. Moreover, no controls are provided from normal human cerebral arteries, minimizing the authors' conclusion that myofibroblasts are unique to cerebral arteries in spasm. The authors failed to grow medial smooth muscle cells from normal cerebral artery in the MfPCL system. It is known that many types of cells can alter their phenotypes to express contractile proteins when the need to contract tissue arises (105). Smooth muscle cells can change their phenotypes and become migratory, losing their ability to express α-actin, the active contractile element in their cytoplasm. They can also change their characteristics in response to culture *in vitro* and injury. In general, mature smooth-muscle cells *in vivo* are characterized by a predominance of α-actin and smooth-muscle isoforms of myosin, presence of desmin, ability to contract, and low rate of mitosis (25,104). When cultured *in vitro*, smooth-muscle cells alter expression of actin so that β-actin predominates and the γ-actin content increases (25,104). This phenotype of smooth muscle also secretes more collagen and noncollagenous glycoprotein than mature smooth muscle (25,188). There are a number of phenotypes of myofibroblasts, some in close resemblance to smooth muscle cells and others in close resemblance to fibroblasts. Thus, the distinction between myofibroblasts and smooth muscle cells could be difficult at times.

Some workers have demonstrated an increase in collagen matrix and presence of fibrosis in vasospastic arteries on quantitative biochemical analysis and ultrastructural studies (16,83,215,298,300). The word "vasospasm" implies active physiological vascular constriction; the proponents of the structural hypothesis have advocated alternative terms such as "acute proliferative vasculopathy" (246), "post-subarachnoid hemorrhage vasculopathy" (3,4), "constrictive angiopathy of subarachnoid hemorrhage" (35,300), and "chronic structural narrowing" of cerebral vessels (92).

However, there is evidence to suggest that cerebral vasospasm cannot be fully explained by the structural theory. Pathological studies reveal that fibrosis develops late after SAH, usually following the period of worst myonecrosis (83,183,199,215). Using semiquantitative (immunofluorescence microscopy) and quantitative (amino acid analysis) methods, MacDonald et al (185) did not notice increases in arterial collagens after SAH except when intimal thickening developed 28 days after SAH. In a primate model of SAH, Findlay and coworkers (83) noted that arterial narrowing and vessel wall thickening in vasospastic arteries were due primarily to medial constriction. Nagasawa et al (215) quantitatively measured the collagen content of dog basilar artery following SAH and found progressive increases that became significant 14 days after SAH. Furthermore, study by Mayberg et al (196) suggested that structural changes in cerebral arteries after SAH do not directly contribute to vessel narrowing through increases in wall mass. Although Smith et al (297,298,300) and other groups (178,296,297) reported qualitative increase of collagen in tunica media of vasospastic arteries under light and electron microscopy, some investigators failed to notice fibrosis in arterial walls of vasospastic arteries from patients with SAH and animal models of SAH (43,69,83,133,253,288). Moreover, since vasospasm usually resolves by two to three weeks following SAH, one would expect fibrosis to do the same if it were holding arteries in a narrowed state. Since arterial narrowing reverses as collagen is deposited within the vessel wall, other processes must be responsible for the chronically narrowed arteries following SAH. In addition, wall stiffening and loss of compliance have not been shown to resolve as luminal narrowing does. Therefore, this "wound-healing" type of fibrotic reaction in the vessel wall cannot completely account for the mechanism of vasospasm. The structural hypothesis is further weakened by the observation that high-dose nicardipine (133) and intra-arterially administered papaverine (148) can reverse cerebral arterial narrowing in vasospasm. The effectiveness of mechanical balloon angioplasty in vasospastic cerebral arteries is not proof of the theory, since forceful mechanical dilatation of vasospastic vessels can potentially paralyze the contractile mechanism of smooth muscle cells as well as disrupt or stretch a fibrotic vessel wall.

c) other hypotheses

Besides the "structural hypothesis" and the "vasoconstrictive hypothesis", another argument, which is less popular than the former two postulates, has been proposed for the pathogenesis of cerebral vasospasm. That inflammatory and immunologic reactions may lead to vasospasm is based on observations that some anti-inflammatory (33) and immunosuppressive (250) agents can reduce vasospasm, and that the subarachnoid space and vasospastic vessel wall are often filled with different types of inflammatory cells (16). However, vasospasm rarely occurs in primary subarachnoid inflammatory conditions, such as purulent meningitis, and this finding argues against the role of inflammation in vasospasm.

Whether chronic vasospasm is purely physiologically based or occurs because of vascular morphological changes remains unclear, due to the lack of a perfect experimental model of subarachnoid hemorrhage. The arguments presented by both sides of the controversy seem to contradict each other. However, at close scrutiny, the seemingly contradictory arguments only serve to illustrate our incomplete understanding of, and the complexity involved in, the pathogenesis of cerebral vasospasm. In the future, all the findings available will likely complement and reconcile with each other, and researchers will probably find that chronic cerebral vasospasm is a result of a combination of physiological and structural, processes, both intra- and extracellular in location.

Morphological changes in vasospastic cerebral arteries

MacDonald et al (183) showed that there is a marked similarity between vasospastic smooth muscle cells and smooth muscle cells from arteries contracted with PGF2α, suggesting that smooth muscle contraction occurs during vasospasm after SAH. Muscle cells of vasospastic arteries and of arteries exposed to oxyhemoglobin, as well as those from arteries contracted with PGF2α, showed markedly convoluted and folded cell membranes (183). Dense bodies which represent attachment sites of intracellular contractile filaments to the cell surface between the folds of membrane have been observed (97,183). Fay and Delise (79) proposed that muscle contraction causes folds of membrane to throw out between dense bodies to accommodate cell cytoplasm displaced by cell shortening. Review of other reports also shows that convolution or corrugation of smooth muscle cell membranes occurs during muscle contraction (12,97,252). However, degenerative ultrastructural changes in the endothelium and media were seen only in SAH-induced, chronic vasospasm, and these changes remained at 14 days despite considerable resolution of arterial narrowing and medial thickening (83,183). Muscular degeneration such as this has been observed in the majority of animal models and human series (3,35,43,49). This observation has raised the uncertainty whether the observed luminal narrowing in vasospastic vessels is attributable to active smooth muscle contraction, and therefore reversible, or due to irreversible structural changes in the vessel wall.

Workers have consistently noted ultrastructural changes as the result of, or coexistent with, vasospasm in human postmortem specimens (43,49,69,133,297) and animal SAH models (3,16,35,71,80,177,199,297). There are a number of similarities between the findings in animal models and those in human autopsies (199). However, animal studies have emphasized electron microscopic findings of the cerebral arteries, usually after perfusion fixation, whereas human studies have been histological after immersion fixation.

Light and electron microscopy studies in human cadaveric cerebral arteries after aneurysmal SAH (43,49,133,297) have demonstrated intimal and medial swelling within a few days post-SAH. Increasing intimal proliferation and necrosis of smooth muscle cells in the media with consequent narrowing of the arterial lumen is noted after 1 to 6 weeks (199). From 3 to 15 months post-SAH, the media becomes fibrotic and luminal narrowing gradually reverses (199). Hughes and Schianchi (133) found that in those patients surviving 17 days or fewer from aneurysm rupture the tunica intima was only slightly swollen, and necrosis of the tunica media as well as irregularities of the tunica elastica were present; in those surviving longer, in addition to medial fibrosis, the intima became the most abnormal component of the arterial wall, showing concentric thickening with fibroblasts, collagen fibers and foamy macrophages. However, it is notable that in the 9 autopsies reviewed by Eldivek et al (69), where no specific arterial abnormalities could be found, all of the patients died within 16 days of aneurysm rupture. The complete absence of intimal thickening on histological examination of some arteries in vasospasm emphasizes that this morphological feature is not a necessary ingredient or product of the arterial narrowing.

Studies in animal SAH models (3,16,17,35,71,80,177,212,297) have frequently revealed early degenerative changes in the endothelial cell layer, including vacuolization, disruption of interendothelial tight junctions, and occasionally endothelial desquamation and luminal microthrombosis. The tunica intima, like the overlying internal elastic lamina, is frequently convoluted due to contraction of the media (83,183). In addition, there is some degree of intimal thickening variously ascribed to edema, polymorph infiltration, granulation tissue, migration (presumably from the media) and proliferation of smooth muscle cells or fibroplasia and collagenization. Alksne et al (3) and others (352) have suggested that cellular proliferation and fibrosis in the intima after SAH may be similar to that seen early in atherogenesis, perhaps indicating a fundamental arterial wall response to various noxious stimuli. Some workers have speculated that endothelial injury, followed by leukocyte or platelet adherence and release of chemotactic and mitogenic factors, may be important in the intimal reaction observed after SAH (36,219). Ultrastructural changes in the endothelium demonstrated in animal models often precede any significant hyperplastic intimal changes evident on light microscopy (83,183). In a study by Findlay et al (83) in a primate model seven days after SAH when arteriographic narrowing is maximal, transmission electron microscopy revealed that endothelial cells were swollen and vacuolized and sometimes lost tight junction with neighbours. The subendothelium was slightly edematous and in places became hypercellular with myointimal cells, but the large increase in vessel wall thickness was due to medial contraction (83).

Fein et al. (80) studied SAH-induced ultrastructural changes of cerebral arteries in rhesus monkeys over a time course of slightly over one week. The authors arbitrarily divided the ultrastructural changes into 3 phases: early spasm (less than 1 day), prolonged spasm (2 to 7 days), and chronic spasm (more than 1 week). The early spasm group showed reduction in the luminal size and corrugation of the IEL. Within 8 hours, lipid figures, lysosomal and mitochondrial degeneration were noted in smooth muscle cells. In the second phase, more muscle cell degeneration with intracytoplasmic vacuoles, loss of tight junctions between endothelial cells with platelet-fibrin thrombus deposition on altered endothelial surface, and nerve fiber degeneration was noted. Finally, more intracellular degeneration in the smooth muscle cells and increased extracellular collagen matrix were present in the chronic phase.

Tanabe et al (325) followed the sequential ultrastructural changes in the cerebral arteries from a canine SAH model up to 24 months after SAH. Within 2 hours after SAH, they noted rounding of endothelial cells, thickening of subendothelial substance, and vacuolation in smooth muscle cells. Increased smooth muscle-like cells (so-called myointimal cells or myofibroblasts) in the intima, and more dense bodies and vacuoles in smooth muscle cells were apparent at 6 hours. These changes as well as intimal thickening were more prominent at 48 hours after SAH. Detachment of some endothelial cells from each other and from the IEL, and degeneration of more muscle cells, could be seen between 3 to 7 days after SAH. At 1 month after SAH, the endothelium returned to normal but intimal thickening was still apparent. The most striking finding at this time was the widespread increase in collagen matrix in the media. At 4 months, the intima nearly returned to normal except for slight subendothelial thickening, and the smooth muscle population in the media was significantly reduced. At 12 months, the intima was unchanged. Some remnants of intracellular lysosome-like dense bodies in many of the smooth muscle cells could still be noted in the media which was otherwise normal. At 24 months after SAH, the intima looked normal and the media would have appeared normal if not for the presence of the occasional dense bodies in the smooth muscle cells and in the extracellular space.

Many other studies in animal models (3,35,177,196,199) have noted a similar initial evolution in arterial wall morphology 3 to 14 days after SAH, and regression to normal from 3 to 6 months or longer after SAH.

Characteristic of vasospastic vessels with narrowed lumen is a markedly thickened media, a change erroneously attributed to either an inflammatory or hypertrophic reaction within this layer of the arterial wall. Morphometric analysis can provide quantitation of the nature and degree of alteration in vessel wall after

SAH. Several studies (83,199) have demonstrated that the observed increase in the media after SAH was not associated with a proportional increase in the volume fraction of this layer; thus, this structural change after SAH probably reflects the configurational alteration related to smooth muscle contraction or rearrangement in extracellular matrix rather than an actual structural mass increase (183,185). The shortening and overlapping of smooth muscle cells in the constricted media could lead to convolution of the underlying intima and elastic lamina (83).

Following SAH, some smooth-muscle like cells seem to migrate to the subendothelial space (between the endothelium and internal elastic lamina) and proliferate there (80,199,325). There is some controversy regarding the origin of these cells. Some workers believe that myofibroblasts originate from fibroblasts in the medial layer (296,297,330), while others believe these cells originate from smooth muscle cells in the media (51,185,295).

In summary, the pathological findings in vasospastic cerebral arteries in both humans and animal models include corrugation and swelling of endothelium, adherence of platelet and fibrin thrombi on the intima denuded of endothelium, occasional separation of endothelium from the underlying basement membrane, corrugation and occasional fracture of internal elastic lamina, rippling of the sarcolemma and vacuolation and swelling and occasional necrosis of smooth muscle cells, proliferation of myointimal cells in the subendothelial space, possibly increased extracellular matrix in the media, and inflammatory infiltrates in the adventitia and perivascular space.

Altered sensitivity to pharmacological agents in vasospastic cerebral arteries

A number of vasoactive substances including oxyhemoglobin, endothelins, noradrenaline, serotonin, prostaglandins, thrombin, potassium and others, have been shown to cause vasoconstriction when applied to cerebral arteries in animal models (16,19,65,157,169,179,180,216,247,255,294,318,332,333,347,379). All of these agonists, except potassium, probably trigger smooth muscle constriction through membrane receptor-operated mechanisms and the phospholipase C pathway decribed earlier. However, these experimental studies have also suggested that vasoconstrictive effects of all of these substances, except oxyhemoglobin, are transient and therefore could not account for the chronic narrowing of cerebral arteries noted in vasospasm.

Both increased and decreased sensitivity of human and animal cerebral vessels to catecholomines after SAH has been reported. Lobato et al (180) noted increased vasoconstrictor response to serotonin in feline posterior communicating arteries after SAH. This heightened sensitivity, as with that displayed in response to noradrenaline, was maximum 3 days after SAH, and slowly decreased afterward. The contractile response to serotonin was still significantly different from that found in controls as long as 4 weeks after the hemorrhage. Pickard and Perry (255) reported a four-fold increase in the contractility of canine vessels 3 days after hemorrhage, although the time course of serotonin hypersensitivity did not parallel the appearance of the delayed spasm in humans (379). Ultrastructural changes, such as disintegration and disappearance of vesicles in the adrenergic terminals (62,70) and an increase in the maximal binding of norepinephrine to adrenergic receptors (337,338), might reflect the basis of impaired and abnormal adventitial adrenergic nerve function (16,67,179,247,318). Loss of catecholamine fluorescence after SAH was demonstrated in basilar arteries of rats (53), rabbits (242,248), and cats (179). Based on these data, denervation hypersensitivity was proposed as a mechanism to explain the intensified response of vasospastic cerebral vessels to catecholamines. However, several studies have shown that in the basilar artery of rabbits (6) and cats (61) it was not possible to demonstrate increased sensitivity to histamine, serotonin, norepinephrine, or potassium after chemical or surgical sympathetic denervation (16).

Other investigators have reported decreased sensitivity of cerebral vessels to catecholamines after SAH in experimental models. Toda et al (332) found that canine blood vessels were mildly hyposensitive to serotonin at 1 and 7 days after experimentally-induced hemorrhage. Simeone et al (294) reported that cerebral vessel sensitivity to serotonin did not change after denervation with 5-hydroxydopamine. Boisvert et al (19) found that injections of blood containing serotonin into the subarachnoid space of monkeys produced only a transient constrictive response. In a monkey model of chronic cerebral vasospasm, Bevan et al (16) showed a marked reduction in the capacity of the vessel wall to contract, and a reduction in constrictor and dilator nerve influences on vascular tone. This was attributed by the authors to increased rigidity of the vessel wall secondary to inflammatory infiltrate and fibrosis. Nagasawa et al (216) reported that the contractile response of spastic arterial wall to serotonin remained unchanged after SAH although spastic constriction increased progressively and became maximal seven days after SAH. They measured the incremental elastic modulus of the canine basilar arteries subjected to experimental SAH, and found that the elastic modulus was significantly lower in the SAH group compared to the control. The elastic modulus correlated well with the ratio of collagen to elastin contents in the post-SAH period.

Despite conflicting reports, several studies suggest that, in general, the response of vasospastic cerebral arteries to vasoconstrictors is reduced. Hypotheses that have been proposed to explain the reduction in the responsiveness include endothelial degeneration, myonecrosis and phenotypic change, proliferative vasculopathy with increased myofibroblasts and extracellular collagen in vessel walls, and an inflammatory infiltration of vessel walls with decreased distensibility (4,34,246,275,325,327). It is also possible that vasospastic vessels are already severely constricted and thus cannot respond as dramatically as nonvasospastic vessels (169). More recently alteration of intracellular signalling of smooth muscle constriction has been increasingly recognized in vasospastic vessels; however, the exact intracellular signal transduction responsible for the decreased response of vasospastic vessels to vasoconstrictive agents is still unclear.

Endothelium-dependent vasorelaxants such as acetylcholine, bradykinin, calcium ionophore A_{23187}, substance P, thrombin, adenosine diphosphate and serotonin, and direct smooth muscle relaxants such as papaverine, PGI_2, atrial natriuritic factor (ANF), calcitonin gene related peptide (CGRP), sodium nitroprusside and nitroglycerin, have been used in the study of vasodilatation in normal and vasospastic cerebral vessels (44,56,73,153,156,210,285,331,354). The mechanism of endothelium-dependent and nonendothelium-dependent vasorelaxation have been described in detail in previous section. Whether there is alteration of cerebrovascular reactivity to vasorelaxants following SAH is not as obvious as in the case of vasoconstrictors; different studies have shown different results (157,169,217,273,331,347). In general, however, most studies have shown that vasorelaxation is impaired in vasospastic vessels.

Kim et al (157) reported that the amount of decrease of *in vitro* endothelium-dependent vasorelaxation was proportional to the degree of *in vivo* vasospasm in a canine SAH model. They showed that the intraluminal release of EDRF (either spontaneous or stimulated by agonists) was not impaired; therefore, the loss of endothelium-dependent relaxations during vasospasm could only be explained either by an abnormal diffion of EDRF, or by the loss of responsiveness of the smooth muscle to the factor. Vorkapic et al (347) showed in a rabbit SAH model that, in addition to reduced contractile response to norepinephrine and potassium, endothelium-dependent vasodilation to acetylcholine was diminished. The highest degree of *in vivo* vasoconstriction was seen when endothelium-dependent vasorelaxation was impaired least. Therefore, loss of endothelial function might enhance the vasoconstrictor influences of substances released from the clot. Endothelium-dependent relaxations in rabbit and dog cerebral arteries mediated via EDRF are abolished by treatment with hemolysate and oxyhemoblobin *in vitro* (190,239) or by subarachnoid injection of blood *in vivo* (157,217). Hemoglobin inhibits endothelium-derived relaxing factor (EDRF) in cerebral as well as systemic arteries. Hemoglobin inhibits the increase in cyclic GMP caused by nitrovasodilators as well as EDRF (137,157,190). Hemoblobin captures nitric oxide (100), which is the active intermediate of nitrovasodilators and is the mediator of endothelium-dependent relaxation. During vasospasm after SAH, hemoglobin is likely to be present in the vicinity of the smooth muscle, since infiltration of blood components is observed in the adventitial layer (177) and disruption of interendothelial junctions also occurs (280).

There are several possible explanations to account for the discrepancies between various studies with regard to the changes in vasorelaxation and vasoconstriction of cerebral vessels following SAH. Interspecies differences and anatomic variations (65,66) undoubtedly play important roles. The canine basilar artery is not necessarily the same as the feline basilar artery, and the basilar artery is not necessarily representative of the entire circle of Willis even in the same species, in terms of vascular sensitivity and maximal response to stimulants (65). It could be that the basis of vasospasm is different in different models and vessels. How changes in experimental models of SAH and vasospasm reflect what is seen in man following aneurysm rupture is not known with certainty.

Management of cerebral vasospasm

In diagnosing vasospasm, it is important to bear in mind its time course. Vasospasm should not be a catch-all diagnosis for all types of neurological worsening in the setting of SAH. The clinical effect of vasospasm depends on a number of factors, the most important being the extent of cerebral arterial narrowing. There is a well recognized but imprecise correlation between the amount of subarachnoid blood detected on initial CT scan and amount of ensuing vasospasm. It should be kept in mind that, independent of arterial narrowing, SAH per se may modify cerebral metabolism and ischemic vulnerability of the brain. This possibility may explain the occasional poor correlation between the extent of angiographic arterial narrowing and the severity of neurological deficit. Transcranial Doppler imaging facilitates bedside detection of developing vasospasm and, therefore, enables prompt intervention prior to damaging cerebral ischemia. Currently, management of cerebral vasospasm falls into 3 main categories - prevention, standard treatment and experimental therapy.

a) Prevention

It is always easier to institute prophylactic measures than to deal with well-established cerebral vasospasm and its devastating consequences. Although the mechanism of cerebral vasospasm is not clearly understood, a few interventions and pharmacological agents have been known to be at least partially effective in ameliorating angiographic narrowing or its associated outcome. These include calcium channel antagonists, mild hypervolemia in the presence of normotension or slight hypertension, 21-aminosteroids, mechanical clot removal by surgery, and clot dissolution by fibrinolytic agents.

Nimodipine, a dihydropyridine, is routinely administered to all patients admitted with SAH. It is a lipophilic calcium channel blocker, and therefore crosses the BBB relatively easily. The Canadian Nimodipine Trial (251) showed that nimodipine treatment was associated with a significantly better outcome (29.2% in nimodipine-treated patients compared to 9.8% of placebo-treated patients). Delayed ischemic deficits (DID) from vasospasm were significantly less frequent in the nimodipine group with permanent deficits occurring in 6.9% of nimodipine-treated patients and 26.8% in placebo-treated.However, angiography showed no significant difference in incidence of moderate or severe diffuse spasm which was seen in 64.6% of nimodipine-treated patients and 66.2% of placebo-treated patients. Pickard et al (254) reported the largest multicenter trial of nimodipine use in SAH patients. The authors did not find any significant reduction of angiographic vasospasm or mortality in the treatment group, although there was better outcome and decreased cerebral infarction. Thus, nimodipine improves outcome and reduces delayed neurological deterioration in patients with SAH by mechanisms other than prevention or reduction of vasospasm as visualized on angiography. Currently, it is still uncertain whether the salutary effect of nimodipine is on vessels, or on neurons protecting them against ischemia(251), or both. It has been proposed that nimodipine improves collateral circulation to the ischemic areas (102).

Routinely, patients with thick subarachnoid blood clot on admission CT scan following aneurysmal SAH are maintained in euvolemia or slight hypervolemia (as well as normotension or slight hypertension) with albumin and crystalloid solutions. The benefit of this measure is a reflection of the gain from "triple-H" therapy, which will be discussed in some detail later. Fluid loading expands intravascular volume, which improves cerebral perfusion pressure and cerebral microcirculation (54,77,149,305,366).

Tirilizad mesylate or compound U74006F (one of the 21-amino steroids) is a potent scavenger of oxygen free radicals and an inhibitor of iron-catalyzed lipid peroxidation, which may play a central role in development of arterial narrowing in vasospasm (91,310), as well as in the final cascade of ischemic cell death (22). In experimental models of SAH, tirilazad was shown by Steinke et al (310) to ameliorate angiographic vasospasm associated with SAH, and by Wilson et al (362) to reduce infarct size in models of focal cerebral ischemia. Hall and Travis (114) demonstrated that tirilazad mesylate retarded development of post-SAH cerebral hypoperfusion in a feline model. Tirilazad is well-tolerated and lacks glucocorticoid side effects in human (147). This drug has undergone preliminary clinical testing. The Phase II Canadian Tirilazad Study (147) compared placebo with tirilazad at doses 0.6, 2.0, and 6.0 mg/kg/day for 10 days following SAH, all patients also receiving nimodipine. In the 245 patients studied, there were no serious adverse medical complication due to the drug. Although the number of subjects in the study was too small to make a statistically sound conclusion, the study suggests that tirilazad in the smaller two doses may reduce vasospasm and improve favorable outcome at three months following SAH.

Since spasmogenic factors most likely represent breakdown products of blood clot in the subarachnoid space, intuitively early operation and removal of the blood clot in the acute stage of SAH may decrease incidence of vasospasm and improve outcome. Experimental studies by Nosko et al (231) and Handa et al (118), and clinical studies by Suzuki et al (315) and other groups (208,326) support this presumption. Despite these encouraging studies, total clot removal from the basal cisterns is technically difficult and poses a real danger to the patients. For these reasons, mechanical clot removal is not a common practice.

To avoid difficulties associated with mechanical clot removal, fibrinolytic agents such as recombinant tissue plasminogen activator (rt-PA) (84-88), urokinase (121,163) and streptokinase (249) have been utillized to lyse subarachnoid blood clot. These agents are usually administered into the basal cisterns following surgical repair of aneurysm. Preliminary data in human (85,207,234,380) and animal models (84,86-88,249,335) have suggested favorable outcome from utilization of fibrinolytic therapy, but a large double-blinded randomized controlled trial is needed to show that this therapy is indeed effective and safe in reducing vasospasm in SAH patients.

b) Standard treatment

Denny-Brown (54) demonstrated in 1951 that hypotension could worsen neurological function in patients with severe structural narrowing of cerebral arteries. A number of other studies have shown that symptoms of cerebral ischemia secondary to insufficient cerebral blood flow could be reversed by volume expansion and/or elevation of systemic blood pressure (11,77,99,149,167, 256,305,366). Improvement in cardiac output by augmentation of preload and increasing cardiac contractility (11,99,149), in addition to decreasing blood viscosity through hemodilution (39,93), have also led to better outcome in patients with vasospasm. All of these treatment modalities share one common feature, which is an increase in cerebral blood flow (CBF). Cerebral blood flow is dependent on cerebral perfusion pressure which, in turn, is directly proportional to systemic blood pressure. In addition, CBF is also affected by blood viscosity. The gold standard treatment for delayed ischemic deficits (DID) secondary to vasospasm is the "triple-H therapy" (hypervolemic hypertensive and hemodilutional therapy) (11,77,99,149,167,305,366). In most circumstances, this involves the use of crystalloid and colloid solutions to increase the circulating blood volume in the patient, lowering the hematocrit to 30 to 35 percent, and elevating systolic blood pressure to 180 to 240 mm Hg. If necessary, cardiac inotropes such as dopamine or norepinephrine are added to elevate blood pressure. This therapy is frequently only partially effective and sometimes ineffective in reversing neurological deficits secondary to vasospasm. It is not without risks (149), especially in patients with unclipped aneurysm (precipitating aneurysm rupture), or in patients with compromised cardiorespiratory status, in whom it can cause pulmonary edema and cardiac failure.

Intraarterial papaverine administration has been used to effectively reverse angiographic vasospasm and neurological deficit (148), but the beneficial effect of this treatment is frequently short-lasting (148).

In summary, currently available therapies are not always effective and are not without risks. Transluminal balloon angioplasty (TBA) has emerged as an increasingly important alternative therapy in symptomatic cerebral vasospasm, particularly in those cases unresponsive to standard therapy.

New treatment of cerebral vasospasm - transluminal balloon angioplasty

a) Historic review of TBA in clinical use

TBA involves mechanical expansion of a narrowed arterial lumen by means of an inflatable microballoon attached to the tip of an arterial catheter. The idea of mechanically dilating a narrowed vessel secondary to a pathological process is not new. In 1964, Dotter and Judkins (60) pioneered transluminal dilatation of stenotic peripheral arteries due to atherosclerosis. Clinical application of their coaxial system and the addition of the latex and caged dilating balloon catheters by Dotter et al and Porstmann led to the development of double-lumen balloon catheters by Gruntzig and Hopff in 1974. Five years later, Gruntzig and his colleagues (108) reported the use of this inflatable balloon catheter in performing percutaneous transluminal angioplasty (PTA) to treat atherosclerotic coronary disease. Stenoses of the abdominal aorta and brachial, axillary, carotid, subclavian, celiac, superior mesenteric, and hypogastric arteries have also been successfully treated with PTA (272). In the early part of 1980's, PTA began as a nonsurgical percutaneous transluminal approach for treatment of nonatherosclerotic lesions, such as renal artery stenosis secondary to fibromuscular dysplasia or Takayasu arteritis (72,272).

Although transluminal mechanical dilatation was used to treat fibromuscular dysplasia of the cervical carotid artery, and atherosclerotic narrowing of both common carotid and vertebral arteries in 1970's, it was not until 1984 that Zubkov et al (384) first reported application of TBA in cerebral vasospasm following aneurysmal SAH. In that report, the majority of 33 patients treated with TBA showed a favorable outcome (384). Shortly after silicone balloon catheter was developed for cerebral angioplasty, Higashida et al. (127) and Newell et al. (223) simultaneously reported the first North American experience in treatment of cerebral vasospasm by TBA in 1989. Since then, a number of other clinical series have described the clinical use of TBA in cerebral vasospasm. Most reports have emphasized that TBA is effective in reversing angiographic and clinical vasospasm, as well as increasing cerebral blood flow to the ischemic areas. The "durability" of TBA for vasospasm has often been inferred from sustained neurological recoveries, and in some series an unspecified number of patients were found to have no recurrence of arterial narrowing on follow-up angiography (64,127,128,130, 172,195,197,222,223,341). In a description of 95 consecutive patients, all of whom underwent control angiography 5 to 7 days following angioplasty, Zubkov et al (382) found no instances of progression of vasospasm in any dilated segment.

Amongst some of the most appealing aspects of TBA are that it is safe in the experienced hands, it is often effective in cases of cerebral vasospasm refractory to maximal medical therapy, and finally, the angiographic effect or result of TBA is impressive and long lasting. However, both the method and indications for cerebral TBA continue to be refined. At the present time TBA is indicated for severe symptomatic vasospasm in the absence of cerebral infarction, but following aneurysm repair, when standard hypervolemic and hypertensive therapy has failed to reverse ischemic neurological deficits. The technique is successful in about three-quarters of the selected patients (13,48,127,171,172,195,197,223,341,382-384), clinical improvement is prompt, and the effect appears long-lasting. Nevertheless, there are several complications (176,223) associated with TBA as a therapy for cerebral vasospasm. There is a danger of reperfusion hemorrhage (223).

CT scan should always be obtained prior to performing this procedure to rule out cerebral infarction, and TBA should be instituted as soon as the patient has become unresponsive to the standard therapy. This procedure should not be performed when cerebral infarction is evident on CT scan because it significantly increases the risk of reperfusion hemorrhage.

Another potential risk of TBA is vessel rupture during balloon dilatation. Fortunately, this is relatively rare because the balloon catheter usually used for cerebral angioplasty is made of silicone elastomer which is gentle and gradual in expansion, therefore avoiding sudden distention and a sudden increase in wall tension which can precipitate vessel rupture. The small size of the cerebral angioplasty catheter (3.5 x 12.5 mm inflated) confers further protection against vessel rupture. The pressure exerted by this type of balloon is usually in the range of 0.5-1.5 atmospheres (370), which is relatively low compared to 5-8 atmospheric pressures exerted by polyethylene balloons used for coronary angioplasty (370). The silicone balloon also elongates and conforms to the luminal shape of the artery.

A third potential risk of TBA is re-rupture of an unclipped or incompletely secured aneurysm distal to the dilated segment. The untreated or partially treated aneurysm may be partially protected from hemodynamic forces and rebleeding by vasospasm in the proximal parent artery. Dilating this vessel might augment flow into the aneurysm and precipitate re-rupture. Linskey et al (176) reported a case of fatal rupture of the intracranial internal carotid artery during transluminal angioplasty for vasospasm, and the authors concluded the rupture probably occurred at the unclipped residual aneurysmal neck where wall structure was the weakest. The more easily accessible cerebral arteries for transluminal balloon angioplasty are the internal carotid artery, basilar artery and the M_1 portion of middle cerebral artery. Several reports have described refinement of cerebral balloon catheters which improve the steerability of the catheter, and modification of techniques to improve accessibility to more distal and selective branches (129,329). For instance, some of the modifications of basic techniques are "wire-in-balloon" and "wire-through-leak balloon" procedures as described by Brothers and Holgate

(24). The "wire-in-balloon" method requires no alteration of the basic balloon catheter/wire system, but rather a change in the way it is used. When a distally curved wire is advanced into a deflated balloon, the balloon and wire tip will form a curve because of the elastic pull of the balloon. The radius of this curve is changed as more wire is advanced. The balloon can be steered by rotating the wire, and it can tolerate the internal pressure from the wire. The result is a steerable balloon tip with variable stiffness and curvature. For the "wire-through-leak balloon" technique, a hole is made in the center of the distal end of a contrast-filled balloon and a distally curved guidewire is inserted protruding through the hole. When the catheter does not advance easily, the balloon should be inflated where it lies at the origin of a branch, or at the proximal end of a luminal narrowing, to dilate that arterial segment. This slight dilatation may facilitate subsequent engagement and entry of the narrowed segment by the balloon catheter. Terada et al described a new microballoon, which is 2.2 x 6.5 mm in diameter when inflated with 0.02 mL of fluid, for use in the M2, A1 and A2 portions of cerebral arteries (329). This balloon, which is much smaller than the conventionally used cerebral balloon (3.5 x 12.5 mm diam. when inflated with 0.1 mL fluid) designed by Interventional Therapeutics Corporations (ITC).

a) Uncertainties of TBA in cerebral vasospasm

Despite growing clinical use of TBA, there remain a number of unanswered questions about this treatment modality:

1. Why do vasospastic vessels rarely restenose following treatment with TBA?
2. Does TBA cause any damage or alterations to vessel walls, and if so, what are the longterm effects of TBA?
3. Do these alterations in the vessel walls reverse with time?
4. How important are these changes in the vessel walls in maintaining the longlasting dilatation of TBA?
5. Is there any correlation between vessel wall alteration following TBA and longlasting dilatation of TBA?

6. How far distally does one have to dilate a vasospastic vessel in order to achieve the maximal effect of TBA?
7. What is the optimal timing for TBA to be most beneficial?
8. What are the indications and precise contraindications of TBA in cerebral vasospasm?
9. How can one further improve accessibility of a branched vessel at an acute angle to a major vessel with a balloon catheter?

c) Mechanism of action of TBA in cerebral vasospasm

Researchers have speculated on the mechanism of action of TBA in cerebral vasospasm based on the findings from electron or light microscopic analyses of morphological changes in vessel walls following TBA in human specimens or animal models.

There are 3 major explanations that could possibly account for the effectiveness and low recurrence of arterial narrowing after TBA in cerebral vasospasm: a) contractile machinery of smooth muscle cells in the vessel wall is impaired by TBA, and the vessel wall therefore can no longer contract; b) extracellular matrices, which are thought to be important in transmitting contractile force from smooth muscle cells to the entire vessel wall, are altered and this rearrangement of extracellular connective tissues could render the entire vessel wall noncontractile; c) mechanical force of TBA physically disconnects smooth muscle cells from the surrounding basement membranes and connective tissue, such that contractile force from each individual smooth muscle cell cannot act in unison with those from other smooth muscle cells to generate effective contraction in the vessel wall. Finally, a combination of these three processes could be responsible for the mechanism of TBA in cerebral vasospasm.

Morphological and pharmacological changes in noncerebral vessels dilated with TBA

The purpose of reviewing the pharmacological and structural effects of TBA in extracerebral vessels is to: a) compare changes in cerebral and extracerebral vessels after TBA; b) anticipate effects of TBA in vasospastic and nonvasospastic cerebral vessels; and c) use the data from extracerebral vessels to support or refute the mechanism of action of cerebral TBA arrived at through analyses of the pharmacological and morphological changes in cerebral vessels treated with TBA.

Little is known about pharmacological changes and structural alteration in cerebral vessels treated with TBA. In contrast, pharmacological and morphological effects of TBA in extracerebral vessels are well known. These effects were mostly studied by researchers investigating the mechanism of action of coronary TBA.

Morphology of atherosclerotic plaque is well-known (268,269,381). The plaques consist of cholesterol crystals and a large amount of collagen, while their luminal surface is covered with endothelium. The number of intimal smooth muscle cells is increased and many contain lipid droplets ("foam cells"). The internal elastic lamina is irregularly stretched and in some areas missing altogether. The tunica media is composed of increased intercellular ground substance and collagen, particularly in the inner layers, while the elastic fibers are irregularly arranged compared to the nonatherosclerotic vessels. The medial smooth muscle cells contain lipid droplets and/or degenerative changes, including swollen mitochondria.

Autopsies of human atherosclerotic coronary vessels treated with angioplasty (181,182,201,209,278,313), and coronary angioplasty in animals (18,78,243,278), have revealed various morphometric findings. These include deposition of fibrin and platelets over denuded endothelium, tearing or cracking of atheromatous intima, dehiscence of the plaque and intima from the underlying media, stretching and tearing of the media, and stretching of the adventitia (1,18,78,201,243,278,313). It is the disruption of the atheromatous plaque and the stretching of the media and adventitia that account for the dilatation of the vessel lumen, and the potential for an intimal flap (201). Therefore, a successful coronary angioplasty can be viewed as a "controlled injury" to the vessel wall (1,201), and overdistention can cause harmful dissection in the media and adventitia and even vessel rupture. In a rabbit model, Zollikofer et al (381) studied the correlation between histological changes in vessel wall, balloon size, and balloon inflation time in both normal and atherosclerotic rabbit aortas after angioplasty. They noted that disruptive changes in normal aorta correlated in a linear fashion with duration of inflation and size of the balloon. On the other hand, no direct correlation between the ultrastructural changes and the 2 parameters of angioplasty was noted in atherosclerotic aortas. The authors attributed the poor correlation in the atherosclerotic vessels to the variation in distribution and thickness of the plaques. The underlying media of the thick plaque remained unaffected, but the vessel wall between the plaques became stretched in atherosclerotic aortas after angioplasty. Unlike atherosclerotic vessel, normal vessels which underwent balloon angioplasty showed uniform stretching of the entire wall.

Angioplasty has also been used to treat other pathological processes other than coronary artery disease. Narrowing due to fibromuscular dysplasia dilates easily with TBA, suggesting a concentric stretching and some shearing of the fibrous tissue, which then heals in its dilated state (72,272). Intimal fibroplasia may be eccentric, and concentric stretching during dilatation may not be possible, leading to less satisfactory results. On the other hand, radiation-induced arterial stenosis involves periarterial fibosis and arterial wall thickening, and TBA is less effective in these cases (109).

Investigators started to examine vasoreactivity impairment in vessels dilated with TBA early in 1980's. Using dogs and rabbits, Castaneda-Zuniga et al (28) showed that *in vivo* TBA caused immediate and permanent damage to the muscular component of the media. They used Gruntzig balloon catheters to dilate normal canine carotid arteries and aortas of rabbits that had been fed a diet containing 2% cholesterol for 8 weeks, and then examined the immediate and delayed (at 2 months after TBA) pharmacological and structural effects in these vessels. Immediate damage to the muscular component of the media was demonstrated by lack of response to vasoconstrictive stimuli (vasopressin). Electron microscopic study revealed extensive fragmentation of the muscle cells immediately following dilatation. The pharmacological impairment seemed to be permanent, as evidenced by the lack of constriction in these vessels 2 months after dilatation. The damaged smooth muscle cells were removed by macrophages and replaced by collagen tissue. The workers concluded that paralysis of the muscular layer of the media is part of the mechanism of ballon dilatation.

The experiment by Wolf and Lentini (367) further confirmed that vessels stretched by *in vitro* balloon dilatation decrease their response to vasoconstrictors, such as potassium and norepinephrine. Stretching vessels between 130-190% of their resting lengths enhanced vasoconstrictor response. However, arteries stretched between 190-220% of resting length showed a significant fall in developed tension in response to vasoconstrictors. These workers also examined the effect of *in vivo* balloon angioplasty in normal rabbit aortas (368), and noted that vasoconstrictor response was attenuated in proportion to the effective stretch applied to the aorta. When stretched beyond 15% of the original diameters, all arteries showed significantly decreased or abolished response to vasoconstrictor challenge. The authors concluded that effective angioplasty induces paralysis of vascular smooth muscle. The study by Schweitzer et al (287) also supports the notion that spontaneous and pharmacologically-induced vasoconstrictive responses (receptor-mediated and voltage-mediated) are attenuated by balloon angioplasty. However, other investigators have shown that blood vessels stretched *in vitro* increase their response to vasoconstrictors (258,324). The discordant results are probably due to the different amount of stretch applied, since it is known that small stretches enhance the vasoconstrictor response, but larger stretches significantly attenuate the contraction induced by either depolarizing agents (potassium) or receptor-mediated agonists (norepinephrine) (367).

Consigny et al (41,42) performed *in-vitro* angioplasty on nonathersclerotic rabbit external iliac arteries with a balloon catheter. The vessels were dilated to 127 -130% of their original diameters for either 20 seconds or 2 mimutes. They showed no difference in luminal diameters when measured angiographically prior to and following TBA. However, vasoconstriction with 70 mM KCl was reduced in the group dilated for 20 seconds, and this constriction was diminished even further with 2 minutes of dilatation. Both short and long dilatations resulted in similar biomechanic changes in the vessel wall, namely decreased wall thickness, increased elastic modulus, and increased circumferential wall stress (41). It is concluded that inflating the vessels for 2 minutes offers no mechanical advantage but produces more smooth muscle cell dysfunction, which may reduce vasospasm and restenosis after angioplasty.

The majority of the studies examined the immediate changes in vessels after TBA. The delayed effects of TBA, however, were relatively unknown. Weidinger et al (356) investigated the vasodilator function of endothelium that regenerated after balloon angioplasty and the relation of this function to the extent of vascular injury and to subsequent intimal proliferation in iliac arteries of New Zealand White rabbits. Vascular injuries were examined *in vitro* 2 and 4 weeks after a "severe" injury (3.0 mm balloon) or a "moderate" injury (2.5 mm balloon). Both degrees of balloon injury caused complete endothelial denudation. Endothelium regrowth 2 weeks after either injury was confirmed histologically and by immunocytochemical staining. The regenerated cells had irregular sizes and polygonal shapes and lacked the typical alignment in the direction of blood flow. Endothelium-dependent relaxation of balloon-injured vessels to acetylcholine and the calcium ionophore A_{231287} were reduced at 2 and 4 weeks after severe injury. After moderate injury, endothelium-dependent relaxations to these agents were reduced at 2 weeks but had normalized by 4 weeks. Endothelium-independent relaxation to sodium nitroprusside, however, was preserved in all study groups. Morphometric analysis revealed an inverse correlation between the degree of intimal thickening and maximal relaxation to acetylcholine. Thus, there is a persistent attenuation of receptor- and nonreceptor-mediated endothelium-dependent relaxations after arterial injury. The degree and duration of endothelial dysfunction depends on the severity of the initial injury, the time lapse since the injury, and is related to the extent of intimal thickness. Because intimal proliferation was greater after severe than after moderate injury, a partial explanation for this finding is that the thickened intima formed a physical or a functional barrier to the actions of EDRF (356). This factor has a short half-life that can be reduced further by superoxide anion (271), a product of macrophages (218), that may be present at sites of vascular injury (268). Smooth muscle proliferation progressed despite regeneration of the endothelium, and there was a significant correlation between degree of smooth muscle proliferation and loss of endothelium-dependent vasodilation (356). These observations indicate that the endothelium regenerating after balloon angioplasty may be dysfunctional with respect to its inhibition of vascular tone and regrowth. Since nitrovasodilators are known to inhibit proliferation of vascular smooth muscle *in vitro*, reduced release of EDRF from the aberrant regenerating endothelium could contribute to intimal and medial smooth muscle proliferation after endothelial injury (356).

Cartier et al (27) performed balloon endothelial denudation of thoracic and abdominal aortas in rats to study the time course and extent of recovery of endothelium-dependent contractions and relaxations after direct arterial injury. They examined smooth muscle function and endothelium-dependent responses *in vitro* at 1, 2, 4, and 8 weeks after aorta injury. At each study interval during endothelial cell regeneration, vascular smooth muscle contracted and relaxed normally to direct stimulation with norepinephrine and sodium nitroprusside. Endothelium-dependent contractions to serotonin returned to normal at 1 week and developed into a hypercontractile response at 8 weeks. Endothelium-dependent relaxations to acetylcholine returned to normal at 8 weeks, but endothelium-dependent relaxations to adenosine diphosphate remained impaired. These experiments demonstrated that regenerating endothelium regained ability to produce contracting factor before relaxing factor, and it even exhibited potentiated contractile activity 8 weeks after injury. Thus, after direct arterial injury, regenerating endothelium has abnormal endothelium-dependent function that could predispose the vessel to vasospasm and thrombosis. However, it is well known that vasospastic cerebral vessels treated with TBA rarely develop recurrence of luminal narrowing within a few months of mechanical dilatation; hence, the results and conclusions of the studies by Cartier et al (27) and Weidinger et al (356) may not be applicable to cerebral TBA.

The vascular endothelium releases endothelium-derived relaxing factor (EDRF), which is now known to be nitric oxide (NO). Nitric oxide relaxes vascular smooth muscle and inhibits platelet aggregation (259) and adhesion in normal blood vessel (302). However, vascular endothelium can also produce contracting factors such as superoxide anion (340) and the polypeptide endothelin (374), which mediate vasoconstriction. Superoxide anion can cause direct constriction of vascular smooth muscle (340) and has been identified as the possible mediator of endothelium-dependent contractions to arachidonic acid in the canine cerebral artry (340). Oxygen-derived free radicals also inactivate EDRF (270). Thus, the cumulative effect of superoxide anion would be scavenging of EDRF and direct contraction of the vascular smooth muscle. In conditions that cause endothelial cell injury or regeneration, there is a selective impairment in the release of EDRF, while the production of contractile factor is maintained or enhanced (27). This is the case after acute coronary occlusion and reperfusion (245), cerebral vasospasm (156,157), atherosclerosis (124,292), and intimal regeneration after mechanical trauma (291).

All of the studies mentioned above have contributed to our current understanding of how TBA works in coronary atherosclerosis. However, one cannot extrapolate the mechanism of action of coronary angioplasty to that of cerebral angioplasty because the pathogenesis of atherosclerotic stenosis is different from that of cerebral vasospasm. In addition, the biomechanical profile of polyethylene balloon catheter used in coronary angioplasty is quite different from that of silicone balloon catheter used in cerebral angioplasty. Despite these differences, the results of the studies in noncerebral vessels will still prove valuable in understanding cerebral TBA. These studies confirm the hypotheses that effective dilatation by TBA might be due to smooth muscle paralysis and/or structural disruption in the vessel wall.

Morphological and pharmacological changes in cerebral vessels dilated with TBA

There are a limited number of reports in the literature regarding the morphological and, especially, pharmacological effects of TBA in cerebral vessels.

Pile-Spellman et al (257) were the first to report the histological and pharmacological changes in cerebral vessels following angioplasty. In this study, *in vitro* angioplasty with a latex balloon, 5 x 2.5 mm in diameter when inflated, was used on isolated normal canine basilar arteries. The vessels were divided into two groups, one undergoing more vigorous dilatation than the other. When the vessels were dilated to 150-200% of their original diameters with 1-3 atmospheric pressure for 20-40 seconds, light microscopy showed disruption of the adventitia, media and intima. These dilated vessels did not respond to 50 mM KCl. However, when the vessels were dilated to 100-104 % of their original diameters, only mild histological changes were noted, including areas of endothelial desquamation, occasional fracturing of the IEL, and thinning of the tunica media. Integrity of the IEL at origins of perforators was routinely conserved. Pharmacologically, these vessels showed mild endothelial dysfunction as evidenced by a decrease in vasorelaxation to substance P. With repeated or prolonged gentler dilatation, endothelium-dependent relaxation of the vessels seemed to be even more impaired although vasoconstriction to UTP or KCl was unchanged. After being preconstricted to 67% of their original diameters with 20 mM KCl , the vessels dilated with TBA showed no reaction to KCl or UTP.These authors showed that vigorous angioplasty (dilatation to greater than 150% of the original diameter) of cerebral vessels could cause structural damage in all 3 layers, and that the degree of structural damage of vessel wall was a function of the forcefulness of balloon dilatation.

Chavez et al (31) performed *in vivo* TBA in normal canine basilar arteries. They dilated the vessels with a silicone balloon to 130% of original diameter at 1.5 atmospheric pressure. The author and his colleagues found areas of endothelial denudation with deposition of fibrin over the damaged endothelium, altered or "corkscrew-shaped" nuclei in smooth muscle cells, as well as stretching and focal dehiscense in the IEL. The changes in the endothelial cells spontaneously reversed completely by day 7 post-angioplasty, whereas the media and IEL still showed mild residual chronic changes at that time. This study raises the question whether angioplasty caused reversible damage, and suggests that smooth muscle alteration may be responsible for the mechanism of action of TBA in cerebral vasospasm. The structural alteration appeared similar but less severe than those observed in coronary arteries following angioplasty.

Yamamoto et al (370) examined the effects of *in vivo* angioplasty in normal cat femoral arteries, and the effects of in-vitro angioplasty in cadaveric human middle cerebral arteries. Polyethylene balloons (2.5 x 10 mm when inflated) were used to dilate the vessels at 1.5 atmospheric pressure for 30 seconds in one group, and at 3 atmospheric pressure for the same duration in another group. Formic acid digestion of all the cellular components exposed the underlying connective tissue matrix, which was examined with scanning electron microscopy. They found that the extracellular collagen fibers and the IEL were slightly stretched at 1.5 atmospheric pressure of dilatation in both cat and human vessels, but were severely stretched and torn at 3 atmospheric pressure. These authors suggest that the longlasting effect of balloon angioplasty is due to disruption of the extracellular connective tissue.

Kobayashi et al (161) studied angioplasty in a primate model. These workers were the only group that examined morphological changes after TBA with TEM and SEM. Seven days following experimental SAH, vasospastic internal carotid arteries dilated with a silicone showed minimal endothelial injury, straightening of corrugation in the IEL and endothelium, and intact smooth muscle cells.

A number of other groups (14,165,384) have also demonstrated morphological changes in vessels dilated with TBA. Morphological changes found after TBA in normal and vasospastic cerebral arteries can vary from mild to severe. In extreme cases, all three layers of the vessel wall were damaged. However, most studies showed only mild changes consisting of flattening of endothelium without any tear, straightening of the IEL with occasional focal dehiscence from underlying tunica media, and no change in the tunica media. Variability between studies is probably related to the extent, duration and pressure of dilatation, the type of balloon catheter used, as well as interspecies and anatomical differences. Polyethylene balloons are designed for coronary or peripheral vessel angioplasty in which a great deal of tension or pressure needs to be generated by the balloon to disrupt an atherosclerotic plaque and dilate an artery narrowed by such a plaque (201). There is a high incidence of complications, such as vessel wall rupture, arterial thrombosis and occlusion and others, associated with the use of polyethylene balloon in coronary angioplasty (201). Acute occlusion or re-stenosis after coronary angioplasty occurs in 2-4 % of cases (201). This is mainly due to either acute vasospasm, thrombosis or plaque dehiscence and dislodgement, causing occlusion (18,28,78,181,243,381). Damaged luminal surface predisposes to platelet adhesion and thrombi formation with release of vasoactive agents causing vasospasm (201). Re-stenosis after successful coronary angioplasty occurs in up to 30% of cases, usually in the first 6 months after angioplasty (201). This is most commonly seen in cases in which the initial stenosis is severe, or in which there is still a high grade of stenosis after the initial angioplasty.

Silicone balloons, on the other hand, are much softer and flexible and elongate and conform to the luminal contour of the vessel. At present, human cerebral angioplasty is routinely performed using silicone balloons. The pressure generated (usually 0.5 - 1.5 atm.) by this type of balloon is usually much less than that achieved with polyethylene balloon (370).

All studies todate examined the post-TBA effects within hours to days of the procedure; longterm effects of angioplasty have not been examined experimentally. Current knowledge of the pharmacological and structural effects of TBA is insufficient to permit a definitive conclusion on the mechanism of action of TBA in cerebral vasospasm. This is partly due to our incomplete understanding of the pathogenesis of cerebral vasospasm, but mostly due to the uncertainty in the relative contribution of functional and structural changes to the effectiveness of angioplasty. More experimental studies are required to further delineate this question.

Summary of the present study

This study was intended to gain better understanding of the mechanism of action of TBA in cerebral vasospasm by examining concurrent pharmacological and morphological changes in basilar arteries in a canine double SAH model. This model was chosen for the present study because it has been well established and the time course of vasospasm following SAH in a canine model approximates that in mankind.

The animals were randomized into two groups, the normal group (n = 10) and the vasospastic group (n = 15). Parallel pharmacological and morphological studies were carried out for these two groups. It was unclear whether SAH-induced changes (pharmacological and morphological) in the vasospastic vessels played any significant role in the durable dilatation observed consistently in clinical practice following TBA for cerebral vasospasm. This uncertainty might be clarified by parallel studies in normal and vasospastic vessels.

In the SAH group, subarachnoid blood was injected on the first day, and again after 48 hours. Baseline cerebral angiography was obtained prior to SAH. Vasospasm was confirmed by a repeat angiography on the seventh day of SAH, and the animals were then killed on the same day, followed by removal of the brains and basilar arteries.

Immediately following harvesting, the brains with the basilar arteries attached were immersed in oxygenated Krebs solution for *in vitro* TBA followed by pharmacological and morphological studies. It should be emphasized that the basilar arteries were still viable in a simulated physiological environment (ie in oxygenated Krebs buffer) while TBA was performed and during pharmacological studies. A sample of each dilated vessel was fixed within an hour for morphological analysis.

In vivo TBA was attempted in a pilot study but was abandoned because of the difficulty in navigating a balloon catheter through tortuous segment of vertebral artery to reach the basilar artery.

Each basilar artery, normal and vasospastic, was divided into two halves, one half treated with TBA while the other half not treated. Each half artery was further divided into two equal segments, one segment used for pharmacological study and the other for morphological analysis. Four groups of vessels were obtained: a) normal vessels without TBA, b) normal vessels with TBA, c) vasospastic vessels without TBA, and d) vasospastic vessels with TBA. All four groups were subjected to the same pharmacological and morphological analyses.

For pharmacological study, changes in vasoconstriction and vasorelaxation following TBA were examined with several vasoconstrictors and vasorelaxants, separately. SEM and TEM were used for the morphological analyses. With SEM, smooth muscle cells and extracellular matrix were examined separately. HCl was used to digest the connective tissue of the vessel walls to expose smooth muscle cells. Samples of extracellular matrix were prepared by treating native vessels with bleach to eliminate smooth muscle cells. The cross-section of each native vessel, with or without TBA, was examined with TEM.

Objectives and Hypotheses

Objectives:

1. *Primary objective:*

Examine the pharmacological and morphological effects of *in vitro* transluminal balloon angioplasty (TBA) in normal and vasospastic canine basilar arteries.

2. *Secondary objectives:*

a) Correlate functional change with morphological change in normal and vasospastic cerebral vessels following TBA.

b) Better understand the mechanism of action of TBA in chronic cerebral vasospasm based on the pharmacological and morphological changes in normal and vasospastic cerebral vessels treated with TBA.

c) Study pharmacological and morphological changes in vasospastic cerebral arteries following SAH (without TBA).

d) Help clarify the pathogenesis of cerebral vasospasm following aneurysmal SAH.

Hypotheses (null)

1. *Primary hypothesis:*

There is no difference in the pharmacological (vasoconstriction and vasorelaxation) and morphological properties between dilated normal and nondilated normal, and between dilated vasospastic and nondilated vasospastic canine basilar arteries.

2. *Secondary hypothesis:*

There is no difference in the pharmacological (vasoconstriction and vasorelaxation) and morphological properties of nondilated vasospastic and nondilated normal canine basilar arteries.

Chapter Two: Materials and Methods

Randomization and ethics

The protocol for this study was evaluated and approved by the University of Alberta Animal and Ethics Review Committee, and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

Twenty-five mongrel dogs between 20 and 36 kilograms were randomized into two groups. Fifteen animals underwent SAH to induce vasospasm, while normal cerebral arteries from the remaining 10 animals served as controls (Figure 7).

SAH group

The canine double hemorrhage model was used (341). Animals were anesthetized with sodium pentobarbital and intubated for all procedures on the day of first SAH, as well as on the third and seventh day following the first SAH. Intermittent boluses of intravenous pentobarbital were given as needed to maintain adequate anesthesia. Before the first SAH, baseline cerebral angiography was obtained , after which 7 ml of autologous arterial blood was injected into the cisterna magna percutaneously after an equivalent amount of CSF was aspirated from the same location. Animals were then tilted at an angle of 60° with their heads down for 45 minutes. Three days after the first SAH, a second SAH was performed in the same manner. At 7 days, vasospasm was confirmed by cerebral angiography, and the animals were killed with a large dose of sodium pentobarbital (30mg/kg). The brain stem with the basilar artery attached was removed within 3 to 4 minutes of sacrifice, and placed in Krebs buffer (120mM NaCl, 5mM KCl, 1.5mM $CaCl_2$, 1mM KH_2PO_4, 1mM $MgSO_4$, 25mM $NaHC0_3$, 5.5 mM dextrose) aerated with 95% 0_2 : 5% $C0_2$ at 37°C, for *in vitro* TBA and studies of contractility. Viability of all samples was confirmed by examining the contractile response to 60 mM KCl (see below).

Animals without SAH but otherwise treated as described, served as controls. Cerebral angiography was not performed in this group.

In vitro balloon angioplasty

Each basilar artery was divided into two segments while still anchored to the brain stem and immersed in oxygenated Krebs buffer. The proximal segment was dilated transluminally with a silicone microballoon catheter (Figure 5), measuring 2 x 4 mm when inflated with 0.01 ml of fluid, and the distal segment was not dilated (Figure 8). The balloon was introduced through a vertebral artery, and then advanced under microscopic guidance into the proximal basilar artery. The proximal half of the basilar artery was dilated twice to approximately 1.5 to 2 times its original diameter along its entire length, for 5 seconds on each occasion, to ensure uniform dilatation. Dilated and nondilated vessel segments were removed from the brain stem, and divided into 2 segments, one for the pharmacological study and the other for electron microscopy.

Groups of vessels for pharmacological and morphological study

Four groups of vessels (Figure 8) were obtained and subjected to the same pharmacological and morphological analyses: a) normal vessels without TBA; b) normal vessels with TBA; c) vasospastic vessels without TBA; and d) vasospastic vessels with TBA.

Measurement of vessel dilatation following in vitro TBA

Vessel dilatation in response to TBA was measured in both normal and vasospastic groups. After TBA, while the vessels were still immersed in Krebs solution, the outer diameter of the artery (y) was measured under light microscopy using a microscopic scale. The outer diameter of corresponding nondilated arterial segments (z) was also measured. The degree of dilatation of the basilar artery in each animal was calculated as (y/z) times 100%. Mean arterial dilatation for each group (normal and vasospastic) was obtained by dividing the sum of all percentage dilatations in that group by the total number of animals in the same group.

Measurement of angiographic vasospasm in SAH group

Basilar artery vasospasm was quantified in the SAH group by comparing baseline arteriographic lumen caliber with the lumen caliber obtained 7 days following SAH (Figure 4). The diameter of the basilar artery was measured at 3 points along the vessel: a) 1 cm distal to its origin, b) midway between the proximal and distal end, and c) 1 cm proximal to termination of the artery. If the initial diameter of the basilar artery at these points is designated a, b, and c, and the diameter of the same artery at these same points on the 7th day following the initial hemorrhage is designated d, e, and f, then percentage spasm on day 7 post-SAH for each individual dog can be calculated as: $(d/a + e/b + f/c)/3 \times 100\%$. An animal was said to have 99% spasm when contrast was no longer visible in the basilar artery on angiography performed 7 days after SAH. Mean angiographic vasospasm for all animals is calculated as sum of mean vasospasm for each animal divided by the total number of animals (see Table 1).

Pharmacological study

Responses of arterial rings were recorded isometrically using force-displacement transducers (Grass FT.03) connected to a Grass model 7D polygraph (both from Grass Instrument Co., Quincy, Mass.). Rings of cerebral arteries were suspended, using two stainless-steel hooks, under a resting tension of 1 gram in organ baths of 10 ml working volume containing Krebs bicarbonate solution maintained at 37°C and bubbled with 95% 0_2:5% $C0_2$. After an equilibration period of 1 hour, during which the Krebs solution was changed every 15 minutes, the response to potassium chloride (60mM) was recorded and preparations were washed until resting tension was again obtained. Cumulative dose-response curves for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-9} to 10^{-6} M), serotonin (5-HT, 10^{-9} to 10^{-5}M), and noradrenaline (NA, 10^{-9} to 10^{-5}M) were then recorded for each arterial ring.

The preparations were preconstricted with $PGF_{2\alpha}$ (10^{-6} M) prior to vasorelaxation studies in which cumulative dose-response curves to bradykinin (BK, 10^{-12} to 10^{-7}M) and the calcium ionophore A_{23187} (10^{-8} to 10^{-5}M) were obtained. Preparations were washed back to resting tension between use of different vasoactive agents.

Electron microscopy

Random segments from the four groups of vessels were examined with transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Cross-sections of the entire vessel wall were examined with TEM. The intact vessel wall, the smooth muscle layer which remained after digestion with hydrochloric acid, and the extracellular matrix which remained after digestion with bleach were examined with SEM.

All specimens were prefixed in 2.5% glutaraldehyde in 0.12M Millonig's buffer solution (pH 7.2) overnight at room temperature. After samples had been washed 3 times, 15 minutes each, in Millonig's buffer, they were postfixed with 1% osmium tetroxide (OsO_4) in the same buffer for 2 hours. These samples were briefly washed in distilled water, and dehydrated in a graded series of ethanol solutions (50%, 60%, 70%, 80%, and 90%: 10-15 minutes each grade) before final two ten-minute rinses with absolute ethanol. From this point onwards, preparation for SEM and TEM samples differed.

For the SEM study, samples in absolute ethanol were dried in a Seevac CO_2 critical-point dryer at 31^0C for 5-10 minutes, and then mounted on aluminum stubs. All samples were sputter-coated with gold (Edwards, Model S150B Sputter Coater) prior to examination under the scanning electron microscope (Hitachi S-2500). To prepare samples of the muscle component of the arterial wall, OsO_4-fixed intact vessel segments were immersed in 8N hydrochloric acid (HCl) at 60°C for 15 to 30 minutes (183,349). Decreasing concentrations of HCl and finally distilled water were used to rinse the specimens, care being taken to preserve the architecture. Specimens were then dehydrated in increasing concentrations of ethanol, dried in a critical-point dryer, and mounted. To obtain preparations of the extracellular components of the vessel wall, intact vessels fixed with OsO_4 were treated with a 0.25% solution of bleach (sodium hypochlorite) at room temperature for 20 to 40 minutes (349). Specimens were then rinsed with distilled water, treated with ethanol, dried in a critical-point dryer, and mounted.

For the TEM study, absolute ethanol bathing the samples was replaced with propylene oxide, which was changed 3 times at 10-minute intervals. Samples were then embedded in Araldite CY212 mixture/propylene oxide for 1 hour, and subsequently in complete Araldite CY212 mixture overnight in a vacuum dessicator. The mixtures embedding the samples were allowed to polymerize at 60°C for 2 days prior to sectioning. Sections were stained with 4% uranyl acetate for 30 minutes and lead citrate for 5 minutes. Photomicrographs of samples were taken at 75 kV using a Hitachi H-7000 transmission electron microscope.

Morphological effects of TBA in normal and vasospastic groups were assessed separately; that is, normal and vasospastic arteries treated with TBA were compared to those not treated with TBA, respectively. Based on electron micrographs, a pair-wise semi-quantitative comparison of morphological differences between arterial segments treated with TBA and those not treated with TBA from each dog was performed by 3 independent individuals blinded to specimen identification. Specific features in scanning electron micrographs were identified as follows: 1) intact vessel wall: degree of luminal narrowing, wall thickness, and corrugation of intima and internal elastic lamina (IEL); 2) smooth muscle cells: degree of cell stretching or rearrangement and cell surface rippling; 3) extracellular connective tissue: degree of fiber stretching and tearing or rearrangement. For TEM of vessel cross-sections, observations included degree of thinning of entire vessel wall and individual vessel wall coats, stretching and breakage of IEL, straightening and stretching and surface rippling of smooth muscle cells, and amount of endothelial changes such as cell loss and flattening of the luminal surface.

Statistical Analysis

For the pharmacological study, comparisons between groups at each concentration for each chemical agent was assessed using one way analysis of variance (ANOVA), followed by a Scheffe's test of multiple comparison if a significant probability was reached (vasoconstrictors and vasorelaxants). Data were expressed as mean ± standard error of the mean. A probability level (p value) of less than 0.05 was considered significant. Computerized programs, Instat 2.1® and Graphad Prizm®, were used to perform statistical analyses and graph data, respectively, in this study. These two software packages were produced by Graphpad Software Incorporated.

Chapter Three: Results

Angiographic vasospasm in SAH group

On the seventh day following the first SAH, angiographic vasospasm in the SAH group ranged from 9 to 99%, with a mean of 54±8% (Table 1).

Vessel dilatation following in vitro TBA

Following dilatation with TBA and removal of intraluminal balloon catheter, the outer diameter of the basilar artery in the normal group was 100 to 157% of the original size, with a mean of 114±6% (Table 2), while that in the SAH group ranged from 100 to 184%, with a mean of 117±5% (Table 3). It should be pointed out that mean post-dilatation diameters in both groups were significantly smaller than actual arterial distention during balloon inflation (150-200% of resting diameters). This finding is due to lack of luminal distention by continuous blood flow as *in vivo*; previous studies suggested that physiologic blood pressure distends normal arteries 150 to 180% of resting length (47).

Vasoconstriction study

Responses to a single dose of KCl (60mM), and cumulative dose response curves for $PGF_{2\alpha}$, 5-HT, and NA were recorded for the 4 groups of vessels (Figure 9). Normal vessels with TBA consistently showed significantly diminished responses to the 4 vasoconstrictors when compared to control vessels not treated with TBA ($p<0.05$). In comparison to normal vessels and vasospastic vessels not treated with TBA, vasospastic vessels treated with TBA were unresponsive to the 4 vasoconstrictors ($p<0.05$). However, for each vasoconstrictor tested, the cumulative dose response curves for normal vessels with TBA and those for vasospastic vessels with TBA were not significantly different. Similarly, the cumulative dose-response curves for non-dilated normal and non-dilated vasospastic arteries were not significantly different for each of the 4 vasoconstrictors, except for $PGF_{2\alpha}$ at 1×10^{-6} M ($p<0.05$) (Figure 9).

In summary, these studies demonstrated that *in vitro* TBA significantly reduced contractile responses of both normal and vasospastic canine basilar arteries, especially the latter. The reduction in response of vasospastic group versus that of nonvasospastic (normal) group were not significantly different.

Vasorelaxation study

Vasorelaxation studies were carried out for 3 groups of vessels: a) normal vessels without TBA, b) normal vessels with TBA, and c) vasospastic vessels without TBA (Figure 2). The fourth group, vasospastic vessels with TBA, could not be employed for vasorelaxation studies since absence of a contractile response in this group precluded precontraction with $PGF_{2\alpha}$, a necessary step for vasorelaxation to be determined.

Compared to normal and vasospastic vessels without TBA, normal vessels treated with TBA showed abolition of vasorelaxation in the presence of either BK or A_{23187} ($p<0.05$) (Figure 10). Compared to normal vessels without TBA, vasospastic vessels without TBA showed no significant difference in response to A_{23187} and BK.

In summary, *in vitro* TBA significantly diminished or abolished endothelium-dependent relaxation of normal canine basilar arteries. No conclusion could be drawn concerning the effects of TBA on vasorelaxation of vasospastic vessels since preconstriction was not possible.

Morphological changes of vasospasm

Features associated with vasospasm were evident under SEM and TEM in all vessels obtained from the SAH group: decreased luminal diameter to wall thickness ratio, corrugation of IEL (without breakage) (Figure 11 and 16A), folding of endothelial surface (Figure 11B, 14C and 16A), and variable constriction and surface rippling (Figure 13B and 16A) of smooth muscle cells were consistently observed.

SEM- changes after TBA

In vitro balloon angioplasty caused similar and consistent morphological changes in both normal and vasospastic vessels. Thus, only photomicrographs of dilated and nondilated vasospastic vessels are shown (Figure 13 and 14). SEM of both normal and vasospastic vessels treated with TBA showed moderate enlargement of the vessel lumen (Figure 14B versus 14A) and patchy endothelial denudation (Figure 14D versus 14C). Following HCl treatment, smooth muscle cells of normal and vasospastic vessels dilated with TBA did not differ from those in nondilated vessels, with the exception that the surface rippling of smooth muscle cells in vasospastic vessels treated with TBA were flattened (Figure 13A versus 13B). On the other hand, the extracellular collagen fibers and elastin of normal and vasospastic vessels treated with TBA did not differ in gross structural arrangement from corresponding nondilated vessels (contrast Figure 13C with Figure 13D, and 13C1 with 13D1). Specifically, fiber breakage was not observed following TBA (Figure 13C and 13C1).

TEM- changes after TBA

Comparison of Figure 15B with Figure 15A illustrates the changes observed in normal vessels dilated with TBA. After TBA, all normal vessels showed: a) flattening of endothelial cells (EDT in Figure 15B); b) straightening, thinning and occasional rupturing of the IEL (Figure 15B); and c) straightening and crowding of smooth muscle cells in the tunica media (Figure 15B).

The morphological effects of TBA in vasospastic vessels are evident by contrasting Figure 16B with Figure 16A. Like normal vessels with TBA, vasospastic vessels treated with TBA consistently showed: a) flattening of endothelial layer (Figure 16B); b) straightening and thinning of the IEL (Figure 16B); and c) stretching and straightening of smooth muscle cells (Figure 16B), more pronounced than that seen in normal vessels treated with TBA. In addition, vasospastic vessels treated with TBA showed decreased surface rippling (Figure 16B) of smooth muscle cells, and thinning of the tunica media (Figure 16B).

Chapter Four: Discussion

Validity of vasospasm model

The present study showed an average spasm of 54% in the SAH group as visualized on angiography. This degree of vasospasm is considered moderate clinically, and is sufficient for the experiments in this study. A thick layer of subarachnoid blood clot overlying the ventral aspect of the brainstem and encasing the basilar artery was noted in most of the animals in the SAH group during brain harvesting, supporting the angiographic finding. Typical morphological features of vasospasm were also evident in this model. Animals were sacrificed on the seventh day of SAH when vasospasm was at its peak (341). Contrary to aneurysmal SAH in humans, none of the animals in the SAH group showed significant symptomatic cerebral ischemia. This finding is presumably due to better collateral circulation in the canine brain, especially in the brainstem where the conducting vessel (basilar artery) is most affected by SAH. Gross pathological examination of canine brains from the SAH group did not reveal any obvious ischemic changes.

Pharmacological and morphological alteration related to vasospasm

Common morphological features of vasospasm such as luminal narrowing, decreased lumen to wall thickness ratio, corrugation of IEL, and frequent rippling of surface membrane in smooth muscle cells were noted in this study (Figure 13B and 16A), consistent with previous publications (83,183,185,351). The cell membrane rippling likely represents smooth muscle contraction. During contraction, actin and myosin of each contractile unit interact and slide past one another, shortening the distance between dense bodies to which actin filaments attach. The excess membranes between the dense bodies then bulge outward, giving the rippled appearance. This surface rippling appeared to be corrected after TBA. Although wall compliance and quantitative analysis of the extracellular matrix proteins were not performed in this study to ascertain presence of fibrosis in the vessel wall, several studies have shown that this proliferative process or wall mass increase does not occur (83,183,185,351). Hence, the arterial narrowing after SAH is best explained by prolonged smooth muscle constriction. Furthermore, the demonstration that intra-arterial administration of some vasodilators can reverse vasospasm (112,148) suggests that active smooth muscle contraction plays a major role in the pathophysiological process of cerebral vasospasm, at least in the early development of arterial narrowing. That TBA causes more profound impairment in the contractility of vasospastic arteries than normal arteries also supports this proposition.

In this study, SAH vessels demonstrated a general trend of decreased reactivity, although not a significant reduction, in both vasoconstriction and endothelium-dependent relaxation experiments. Most previous similar studies showed significant attenuation in response (16,158,169,238,276,332,347). The discrepancy could be related to the species used, the method of induction of vasospasm, and the time after SAH that the vessels were examined. The general tendency towards reduction in contractility of vasospastic arteries can be explained by the fact that smooth muscle can only contract to a limited extent and it is already partly contracted in vasospasm(169).

Mechanism of action of cerebral TBA

The mechanism by which balloon dilatation affects the wall of the cerebral artery and results in sustained luminal widening in cerebral vasospasm is not well understood. The pathophysiology of cerebral vasospasm is very different from that of coronary atherosclerotic stenosis; therefore, the well-understood mechanism of coronary TBA does not apply to cerebral TBA. Unlike coronary and peripheral vascular balloon angioplasty, in which high-pressure balloons crush stenosing atherosclerotic plaques and passively stretch and often tear all three layers of the vessel wall to widen the lumen of the vessel (17,32,201), cerebral TBA uses a soft and low-pressure microballoon to dilate the narrowed cerebral artery. Minimal structural damage is noted in most experimental studies of cerebral TBA, suggesting cerebral TBA works by different mechanism. If vasospasm represents active smooth muscle cell contraction, then sustained arterial dilatation following TBA might denote smooth muscle cell injury and paralysis, which has been observed in experimentally dilated peripheral arteries following TBA (28,47,104,125,258,287,367,368).

Results of the present study suggest that following *in vitro* balloon angioplasty there is a profound functional impairment in vascular reactivity.

Prostaglandin $F_{2\alpha}$, noradrenaline, and serotonin all produce a contraction of smooth muscle cells through activation of membrane receptors in these cells and activation of a complex signal transduction process (228,346,352). In contrast, potassium chloride generates smooth muscle contraction by depolarizing the smooth muscle cell membrane and promoting calcium entry through voltage and dihydropyridine-sensitive calcium channels (68). Thus, the finding that arteries treated with TBA had greatly diminished responses to compounds whose activity depends on receptor-operated mechanisms, suggests that function of the smooth muscle cell membrane is impaired by TBA. The mechanism might involve the prevention of intracellular signalling and actin-myosin cross-bridge formation, and perhaps by interfering with calcium homeostasis.

Vasorelaxation studies in this experiment suggest that TBA can alter endothelial function in regulation of vascular tone. Bradykinin and calcium ionophore, both endothelium-dependent vasorelaxants, act through different mechanisms to stimulate production of nitric oxide (NO), a potent vasodilator. Bradykinin acts on membrane receptors of endothelial cells and, through a complex signal transduction mechanism, promotes increase of cytosolic calcium and subsequent entry of calcium across the plasma membranes of endothelial cells (56,76,159,210). Unlike bradykinin, calcium ionophore directly promotes entry of calcium across the endothelial cell membranes (159). In both cases, elevation of intracellular calcium stimulates nitric oxide synthase to produce nitric oxide (NO), which rapidly diffuses both within the endothelial cells and across membranes to nearby smooth muscle cells to cause relaxation (56,76,159,210). Impairment in any steps involving NO, such as NO synthesis in endothelial cells, diffusion pathway from endothelial cells to smooth muscle cells, or interference of interaction between NO and soluble guanylyl cyclase in smooth muscle cells, may explain the lack of response to endothelium-dependent relaxants in arteries treated with TBA. Although the physiological integrity of the endothelium in vasospastic vessels dilated with TBA could not be readily assessed in this study due to the lack of precontraction with vasoconstrictors, it is reasonable to assume that the endothelium of these vessels probably sustains the same degree of insult and impairment as in normal vessels treated with TBA. This conjecture is supported by previous studies (31,161,165) and the observation in this study that endothelium of vasospastic and normal vessels dilated by TBA underwent similar morphometric changes.

Vasospasm has also been considered to result from structural alteration in the cerebral arterial wall, regardless of the extent of vasoconstriction (83,185,186,199), and it has therefore been suggested that TBA may produce arterial dilatation in vasospastic vessels through passive stretching or rearrangement of extracellular connective tissue. On the other hand, there is evidence that arterial wall thickening in cerebral vasospasm represents vascular contraction, and not cellular proliferation, and this wall thickening by itself does not contribute significantly to luminal narrowing (83,186,199). Therefore, "thinning" of a hyperplastic arterial wall would not appear to be a probable mechanism of action of TBA in vasospasm. It has been recently proposed that chronic vasospasm is due to deposition and contraction of the extracellular matrices of the arterial wall, mediated by myofibroblasts (35,144,296,369,371). These same investigators have also observed stretching and tearing of extracellular collagen fibers in feline femoral arteries and human cadaveric middle cerebral arteries immediately following TBA, and concluded that the long-lasting effects of balloon dilatation may be caused by disruption of connective tissue that proliferates in the vessel wall after SAH (14,370). While we did not observe such changes in extracellular matrix, this discrepancy may be due to a difference in the type of balloon catheter and extent of vessel dilatation employed (41,381). Yamamoto et al (370) used a stiffer polyethylene balloon and higher balloon pressure (about 3 to 6 times that obtained with the silicone balloon), conditions not normally employed in clinical cerebral angioplasty. Thus, the changes observed in our experiment may more closely reflect those associated with human cerebral TBA, and do not provide support for the proposal by Yamamoto et al for the mechanism of action of TBA in cerebral vasospasm.

This study provides the first documentation of concurrent pharmacological and morphological changes after TBA in vasospastic as well as in normal cerebral arteries. In an abstract, Pile-Spellman et al (257) reported the pharmacological effects of *in vivo* TBA in normal canine basilar arteries, and obtained results consistent with our findings. Kobayashi et al (161) performed *in vivo* microballoon angioplasty on vasospastic monkey internal carotid arteries and observed electron microscopic (SEM and TEM) changes similar to those found in our experiments, although endothelial denudation was not observed. Chavez and coworkers (31) studied morphological changes in canine basilar arteries after *in vivo* silicone balloon angioplasty, and their light microscopic findings were consistent with our results. They also noted alteration in smooth muscle cell nuclei, which normalized about 7 days after angioplasty.

Conclusions

Current study has provided some evidence supporting the hypothesis that cerebral vasospasm following SAH is due to smooth muscle constriction. However, the main goal of this study was to examine pharmacological and gross morphological changes produced by acute TBA in cerebral arteries, and the results indicate a greater impairment in vasoreactivity than structural disruption of the vessel wall. Balloon dilatation and stretching of the normal and vasospastic cerebral arterial wall, which can be observed morphologically on electron microscopy, results in a profound alteration in smooth muscle contraction and vasodilatation. Present study favors smooth muscle dysfunction rather than extracellular connective tissue alteration as the cause of vessel dilatation after TBA.

Based on our morphological analysis, it is not certain whether impairment of vascular contraction following TBA is due to: a) disruption of contacts between smooth muscle cells and surrounding connective tissue; b) alteration of membrane receptors; or c) derangement of intracellular pathways of smooth muscle contraction. Interference with any step in the intracellular mechanisms which include intracellular calcium homeostasis, interaction of calcium with calmodulin and myosin light chain kinase, and cross-bridge formation between actin and myosin filaments, may inhibit smooth muscle contraction after TBA. Further studies are needed to differentiate between these possibilities.

Limitations of the present study

It is important to point out that these studies are in an experimental model rather than in human vasospasm. The usual caveats about animal models of clinical disease are applicable. However, the canine double hemorrhage model has been well established for more than ten years, and vessels in spasm from this model bear a strong resemblance to those in human vasospasm, from both a pharmacological and a morphological perspective. It seems probable that the results obtained in our study are relevant to the acute mechanism of *in vivo* cerebral TBA in humans, although more convincing arguments based on longer-term or delayed pharmacological, structural or biochemical responses to TBA cannot be readily produced from the present study. However, there is evidence that longterm smooth muscle paralysis up to two months after TBA can be expected (28). This permanent smooth muscle dysfunction certainly can explain the rare recurrence of arterial narrowing even months to years after TBA in vasospastic cerebral arteries.

This study did not examine submicroscopic structural changes nor biochemical alterations in the extracellular connective tissue of the cerebral arterial wall after TBA. It remains possible that some biochemical perturbation or a subtle disruption in the extracellular matrix of the cerebral arterial wall could impair smooth muscle function in some unknown fashion contributing to vascular paralysis. However, based upon our current knowledge (28,31,47,104,125, 258,287,367,368) and evidence from this study, direct smooth muscle cell dysfunction is a more likely cause of the changes observed in this study.

Chapter Five: Recommendations

This study examined the effects of *in vivo* TBA in normal and vasospastic canine basilar arteries within hours of the procedure. Results of this study have shed some light on the acute mechanism of action of TBA in cerebral vasospasm. However, thorough understanding of this process requires further investigation. The following additional studies may help consolidate the findings and conclusions of this study.

Similar studies using *in vivo* TBA instead of *in vitro* TBA as in this study will be more meaningful. Attempt of *in vivo* TBA in this study was abandoned because of the difficulty in navigating an angioplasty catheter into the canine basilar artery through the tortuous portion of vertebral artery near the lateral vertebral foramen of atlas. The difficulty is partly due to the relative tight fit of balloon catheter in the vertebral artery. However, *in vivo* TBA of canine basilar arteries has been accomplished by others (31). *In vivo* TBA may become easy with experience, modification of technique and angioplasty catheter, and perhaps larger animals with larger vertebral arterial.

The acute changes presented in this study may not necessarily reflect longterm effects of TBA in cerebral vasospasm. These doubts may be clarified by examining cerebral vessels at different time intervals after *in vivo* TBA. The animals can be sacrificed at one-, three-, six-, and twelve-month intervals, and the basilar arteries subjected to similar pharmacological and electron microscopic analyses. In addition, alteration in intracellular contractile proteins can be detected by fluorescent monoclonal antibody binding studies, and quantitative analysis of extracellular matrix protein by immunohistochemical studies can be performed at these intervals. Results from these studies may provide further clues to the mechanism of action of TBA in cerebral vasospasm.

Studies of human cerebral arteries will provide even more convincing results. Analysis of the changes in cerebral vessels in patients who have just succumbed to vasospasm of aneurysmal SAH shortly after TBA will reveal accurate short-term changes induced by TBA. On the other hand, examination of cerebral vessels in patients who die months or years after SAH and cerebral TBA will provide information on the longterm effects of TBA in vasospasm. However, human specimens of this nature are rare and difficult to obtain at present time. Perhaps this type of study will be less formidable in future when cerebral TBA becomes a more common practice.

The hypothesis that smooth muscle dysfunction rather than extracellular matrix alteration dictates the mechanism of TBA in cerebral vasospasm could be strengthened by examination of compliance and cytoskeletal and extracellular matrix protein in arterial wall dilated with TBA. An increase in compliance would imply that vasospastic vessels dilated with TBA do not renarrow because of disruption and weakening of the extracellular matrix in the arterial wall. Arterial wall compliance should remain the same if smooth muscle function alone is changed after TBA. If extracellular matrix is affected by TBA, the amount or ratio of extracellular matrix proteins (collagen, elastin, ground substances) may change during a reparative process. Although vasoreactivity experiments in this study showed dysfunction of smooth muscle and endothelial cells after TBA, it is still unclear if arteries would further dilate with a direct smooth muscle relaxant such as papaverine. The experiment with papaverine may confirm whether smooth muscle dysfunction plays any role in the impairment of endothelium-dependent relaxation after TBA in this study.

These additional studies will settle some uncertainties of TBA in cerebral vasospasm, in addition to strenghtening the primary hypothesis in this study.

Dog no.	proximal (a) %	midpoint (b) %	distal (c) %	mean=(a+b+c)(1/3) %
1	28	21	37	29
2	40	47	48	45
3	18	26	21	22
4	60	57	42	53
5*	99	99	99	99
6	62	59	55	59
7*	99	99	99	99
8*	99	99	99	99
9	38	13	19	23
10	63	62	61	62
11	13	86	58	52
12	17	14	16	20
13	52	44	42	46
14*	99	99	99	99
15	5	13	8	9

Table 1. Distribution of angiographic vasospasm in SAH group. Percent vasospasm at three points along the basilar artery was measured: a= 1 cm distal to its origin; b= midway between the proximal and distal end; and c= 1 cm proximal to termination of the artery. Mean angiographic vasospasm for each animal = (a+b+c)(1/3). *An animal was said to be in 99% spasm when contrast was no longer visible in the basilar artery on angiography performed 7 days after SAH. Mean angiographic vasospasm of all animals = (sum of mean vasospasm for each animal) ÷ (total number of animals) = sum of all readings in the shaded area ÷ 15 = 54 ± 8% (standard error of mean). See Chapter Two for details.

Dog no. (normal group)	% enlargement of external diameter post-TBA (W)
1	110
2	106
3	100
4	101
5	100
6	116
7	112
8	102
9	134
10	157

Table 2. Percent enlargement of vessel external diameters in normal (control) group after TBA. The external diameters were measured using a microscopic scale after balloon catheter was removed from vessel lumens. W = (post-TBA external diameter of vessel) ÷ (pre-TBA external diameter of vessel) x 100%. Mean enlargement of external diameter of basilar artery of all animals = (sum of % enlargement in each animal) ÷ (total number of animals) = 114 ± 6% (standard error of mean). TBA = transluminal balloon angioplasty.

Dog no. (SAH group)	% enlargement of external diameter post-TBA (Y)
1	100
2	114
3	109
4	116
5	104
6	125
7	106
8	112
9	102
10	112
11	117
12	113
13	107
14	139
15	184

Table 3. Percent enlargement of vessel external diameters in the SAH group after TBA. The external diameters were measured using a microscopic scale after the balloon catheter was removed from vessel lumens. Y = (post-TBA external diameter of vessel) ÷ (pre-TBA external diameter of vessel) x 100%. Mean enlargement of external diameter of basilar artery of all animals = (sum of % enlargement in each animal) ÷ (total number of animals) = 117 ± 5% (standard error of mean). TBA = transluminal balloon angioplasty.

Figure 1. Summary of intracellular calcium homeostasis and intracellular mechanism of contraction in a smooth muscle cell. The details of all pathways are described in section "smooth muscle contraction" in Chapter One. Abbreviations and symbols: Ca2+, calcium; $[Ca2+]_i$, intracellular calcium concentration; $[Ca2+]_{SR}$, calcium concentration in SR; CaM, calmodulin; Ca2+.CaM, calcium calmodulin complex; Ca2+.CaM.MLCK, calcium-calmodulin-myosin light chain kinase complex; Cds, caldesmon; Ch_v, voltage-dependent calcium channel; Ch_r, receptor-operated calcium channel; Cp, calponin; DAG, 1,2-diacylglycerol; Ex, Na+/Ca2+ exchanger; G, GTP-binding protein; 5-HT, serotonin; IP3, 1,4,5-trisphosphate; K+, potassium ions; L, ligand; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; Na+, sodium ion; NA, noradrenaline; NaK, Na+/K+-transporting ATPase; PC, phosphotidylcholine; PC-PLC, PC specific PLC; PGF2a, prostaglandin F2a; PI, phosphatidylinositol 4-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phosphoinositide-specific phospholipase C; PLD, phospholipase D; R1, receptor for vasoconstrictive agonists; R2, the SR Ca2+ release channel-IP3 receptor; R3, receptor; SR, sarcoplasmic reticulum.

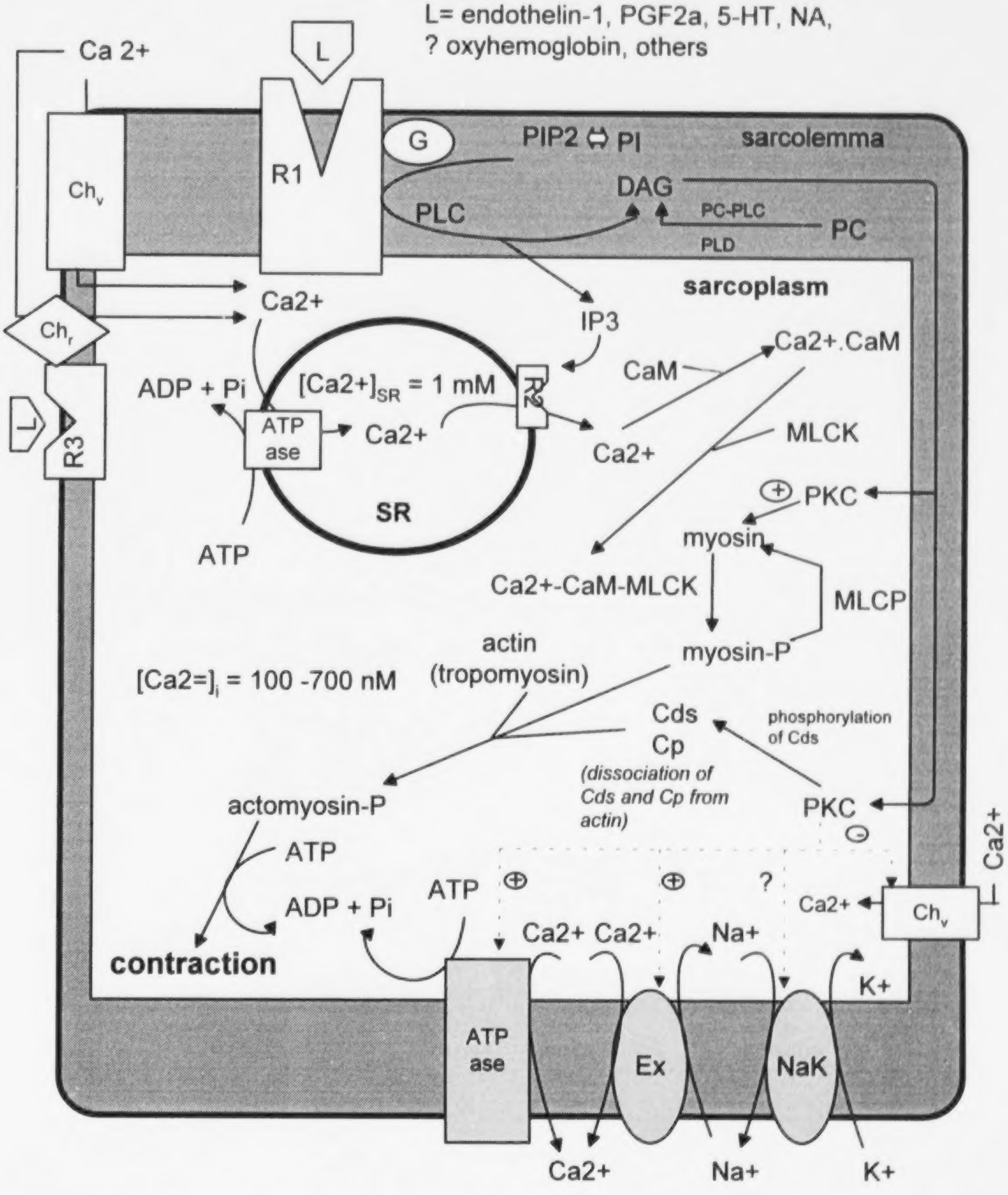
L= endothelin-1, PGF2a, 5-HT, NA,
? oxyhemoglobin, others
Ca 2+
L
G
PIP2 ⇔ PI
sarcolemma
Ch_v
R1
DAG
PLC
PC-PLC
PC
PLD
sarcoplasm
Ca2+
Ch_r
IP3
Ca2+.CaM
CaM
ADP + Pi
$[Ca2+]_{SR}$ = 1 mM
R2
L
R3
ATP ase
Ca2+
Ca2+
MLCK
SR
⊕ PKC
ATP
myosin
Ca2+-CaM-MLCK
MLCP
actin
(tropomyosin)
myosin-P
$[Ca2=]_i$ = 100 -700 nM
Cds
Cp
phosphorylation of Cds
(dissociation of Cds and Cp from actin)
actomyosin-P
PKC
⊖
ATP
Ca2+
ADP + Pi
ATP
⊕
⊕
?
Ca2+
Ch_v
contraction
Ca2+ Ca2+
Na+
K+
ATP ase
Ex
NaK
Ca2+
Na+
K+

Figure 1.

Figure 2: Summary of intracellular mechanism involved in endothelium-dependent relaxation in vascular smooth muscle cell. Endothelium produces prostacyclin (PGI2) and nitric oxide (NO) or endothelium-derived relaxing factor (EDRF) in response to receptor-mediated agonists or calcium ionophore (A23187). Nitric oxide stimulates soluble guanylate cyclase (sGC), resulting in increased cyclic guanosine monophosphate and subsequent smooth muscle cell relaxation. Details of all pathways are discussed in section "Regulation of cerebral vascular tone by endothelium" in Chapter One. Abbreviations: ACh, acetylcholine; BK, bradykinin; R, receptor; PLC, phospholipase C; AC, adenylyl cyclase; DAG, diacylglycerol; IP3, inositol trisphosphate; PGI2, prostaglandin I2; L-arg, L-arginine; cAMP, cyclic adenosine monophosphate.

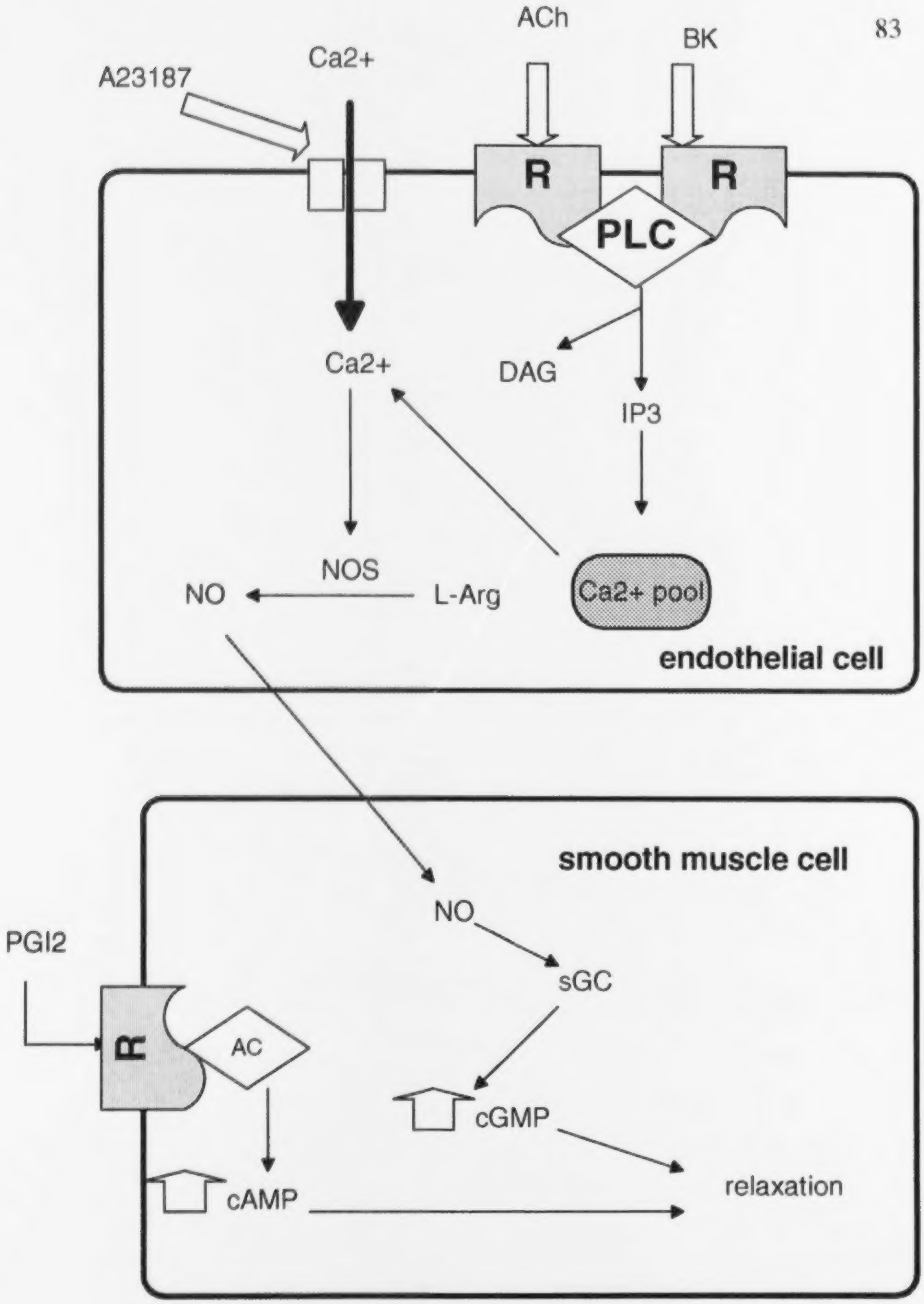
ACh
BK
Ca2+
A23187
R
R
PLC
Ca2+
DAG
IP3
NOS
NO
L-Arg
Ca2+ pool
endothelial cell
smooth muscle cell
NO
PGI2
sGC
R
AC
cGMP
relaxation
cAMP

Figure 2.

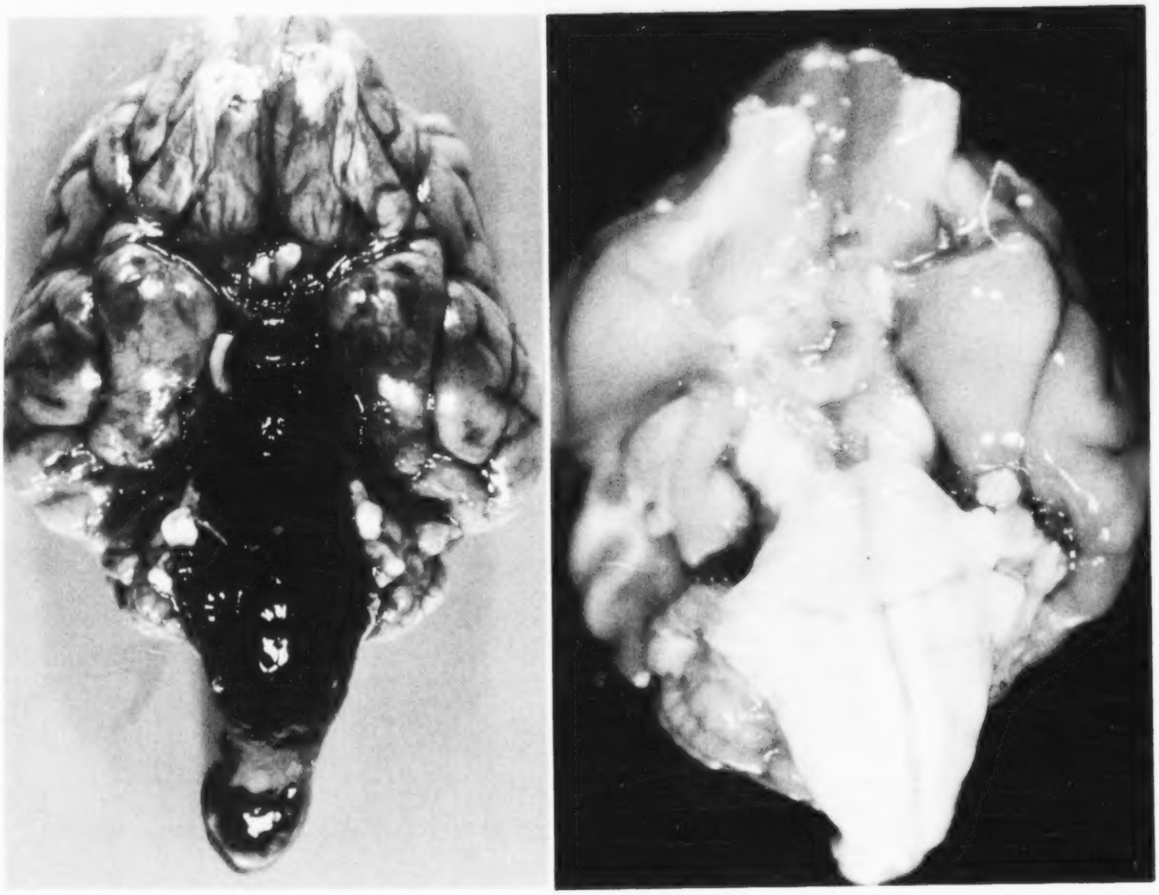

Figure 3. *Left*: Photograph of a canine brain with subarachnoid hemorrhage. A thick layer of blood clot coats the ventral aspect of the brainstem and encases the basilar artery, an ideal situation to produce vasospasm in the basilar artery. ***Right***: Photograph of a normal canine brain. The ventral aspect of the brainstem and basilar artery are free of blood clot.

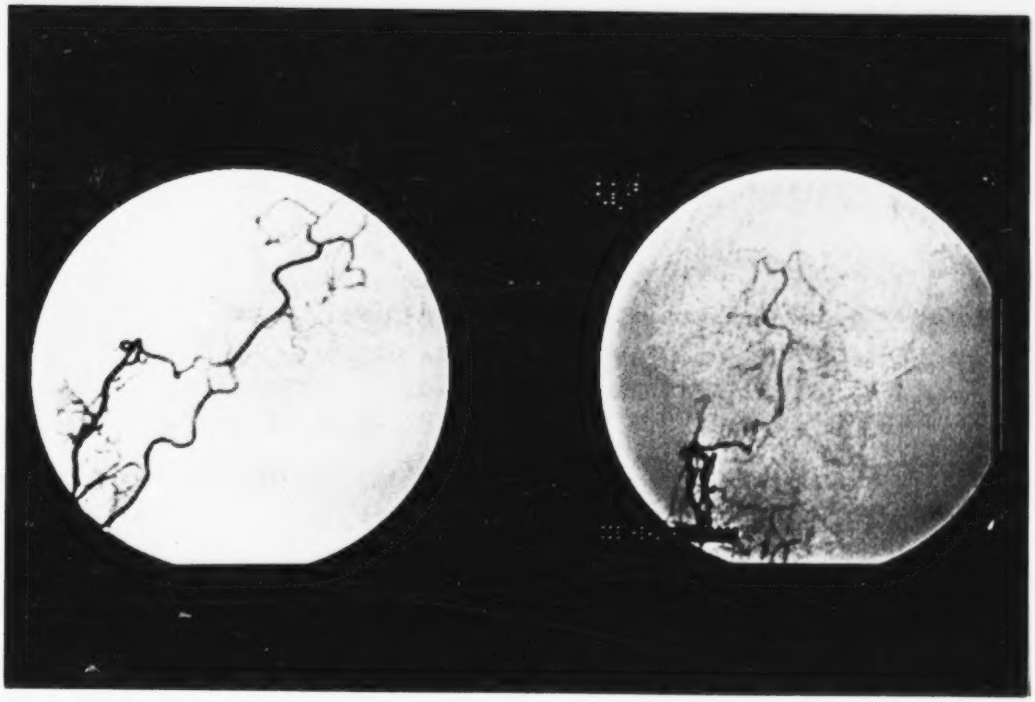

Figure 4. Cerebral angiography of Dog 8 from the SAH group. *Left*: Angiogram taken on day 1 (prior to SAH) showing basilar artery with normal caliber. *Right*: Angiogram taken on day 7 of SAH showing basilar artery with narrowed luminal diameter.

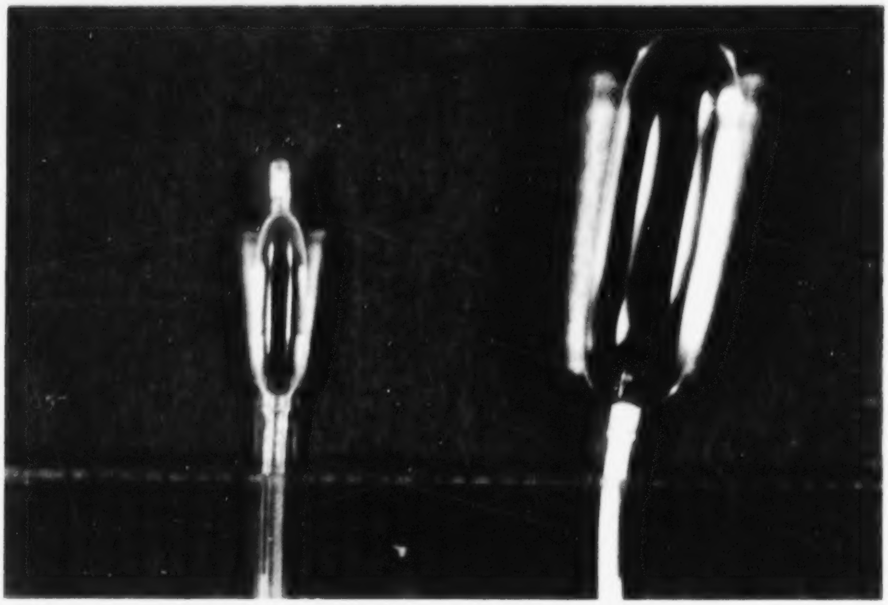

Figure 5. Photograph of silicone balloon catheters (inflated) used for experimental (*left*) and clinical (*right*) cerebral angioplasty. *Left*: Smaller balloon (2.1 mm in diameter when inflated) is used for *in vitro* angioplasty of canine basilar artery in this study. *Right*: Larger balloon (4 mm in diameter when inflated) is routinely used in human cerebral angioplasty. These silicone balloons are soft and flexible; they elongate and conform to luminal contours of blood vessels, reducing risk of vessel rupture.

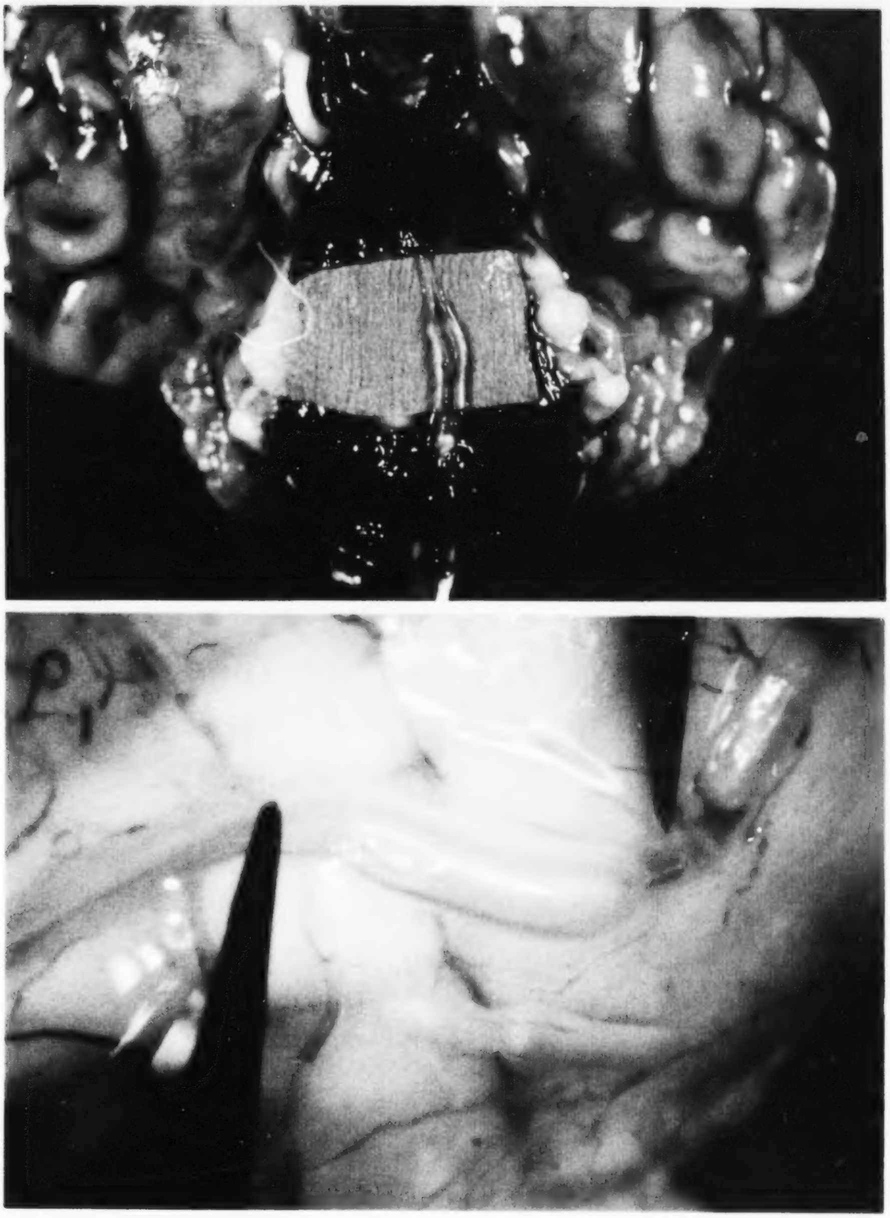

Figure 6. Photographs of *in vitro* transluminal balloon angioplasty in canine brains. *Top*: Angioplasty of canine basilar artery. Balloon is inflated and dilatation of the adjacent vessel segment can be noted. *Bottom*: Close-up view of balloon angioplasty of basilar artery. The balloon is inflated and the arterial segment encircling the balloon appears dilated compared to the nondilated segments distal and proximal to the balloon.

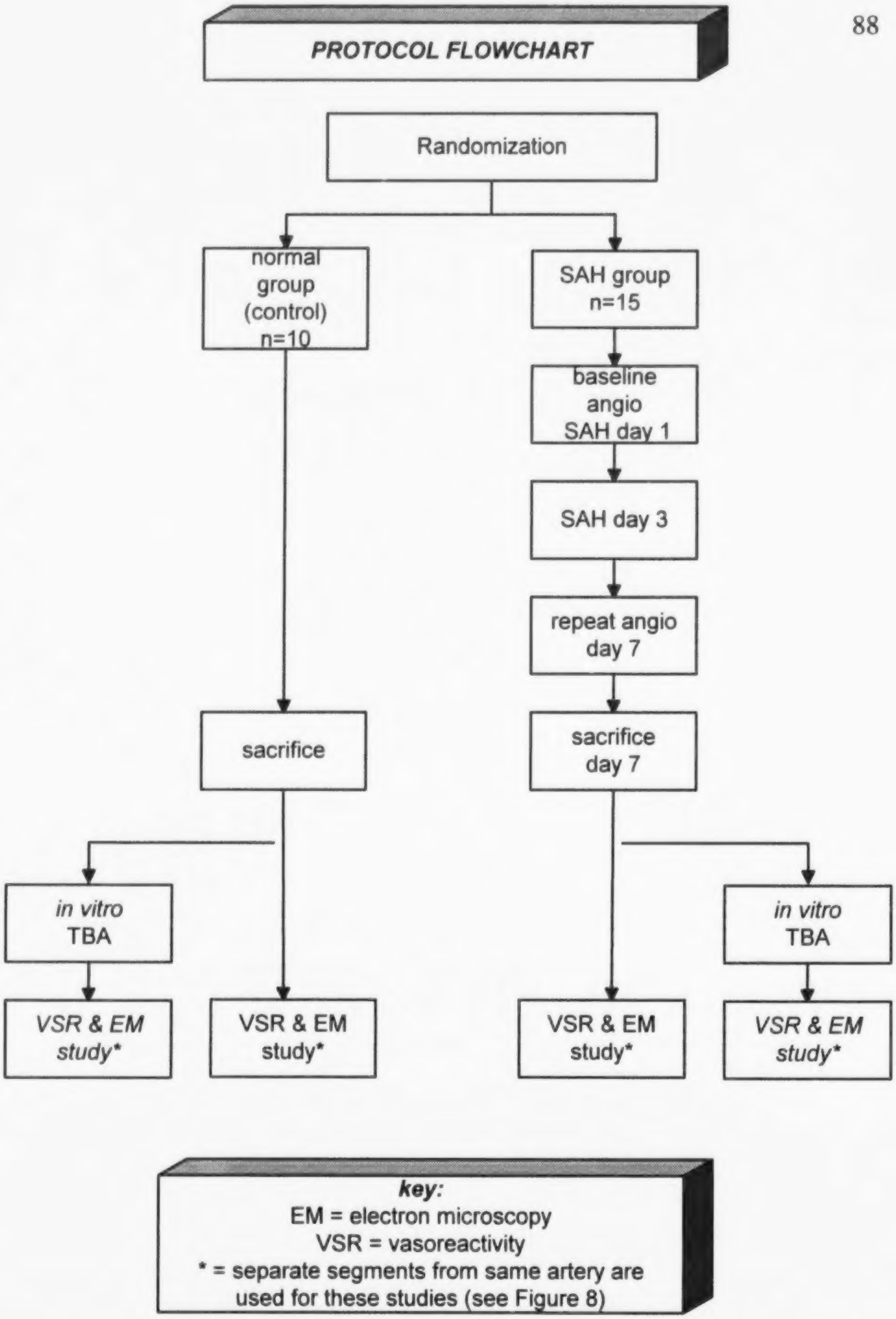
PROTOCOL FLOWCHART
Randomization
normal group (control) n=10
SAH group n=15
baseline angio SAH day 1
SAH day 3
repeat angio day 7
sacrifice
sacrifice day 7
in vitro TBA
VSR & EM study*
VSR & EM study*
VSR & EM study*
in vitro TBA
VSR & EM study*
key:
EM = electron microscopy
VSR = vasoreactivity
* = separate segments from same artery are used for these studies (see Figure 8)

Figure 7. Flowchart showing how arteries in each group are treated prior to morphological and pharmacological studies.

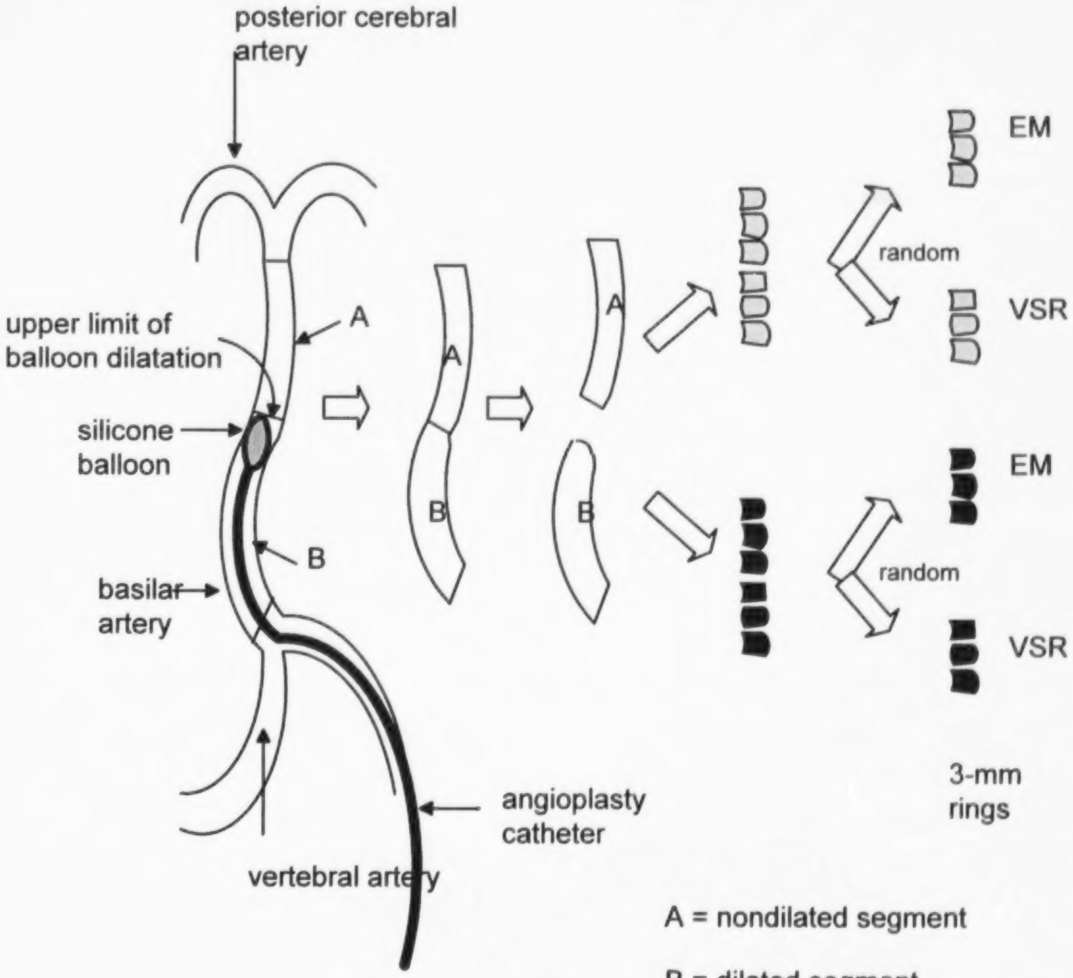
posterior cerebral artery
upper limit of balloon dilatation
silicone balloon
basilar artery
vertebral artery
angioplasty catheter
A
B
A
B
A
B
random
random
EM
VSR
EM
VSR
3-mm rings
A = nondilated segment
B = dilated segment

Figure 8. Schematic diagram showing how each basilar artery was treated and divided into final 3-mm rings for electron microscopic study (EM) and vasoreactivity study (VSR). Note that segment B was dilated with balloon and segment A was nondilated to serve as a control.

Figure 9. Effects of transluminal balloon angioplasty (TBA) in the contractility of normal and vasospastic vessels. Cumulative dose-dependent response curves for 4 groups of vessels: a) normal vessels without TBA (N-TBA); b) normal vessels with TBA (N+TBA); c) vasospastic vessels without TBA (V-TBA); d) vasospastic vessels with TBA (V+TBA). Four different vasoconstrictors were used: potassium chloride (KCl), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), serotonin (5-HT), and noradrenaline (NA). One concentration (60mM) of KCl was tested. In all cases, dilated normal (N+TBA) and dilated vasospastic (V+TBA) vessels showed significant reduction in vasoconstriction compared to non-dilated vessels ($p<0.05$). Dilated vasospastic group did not respond to any of the 4 vasoconstrictors.

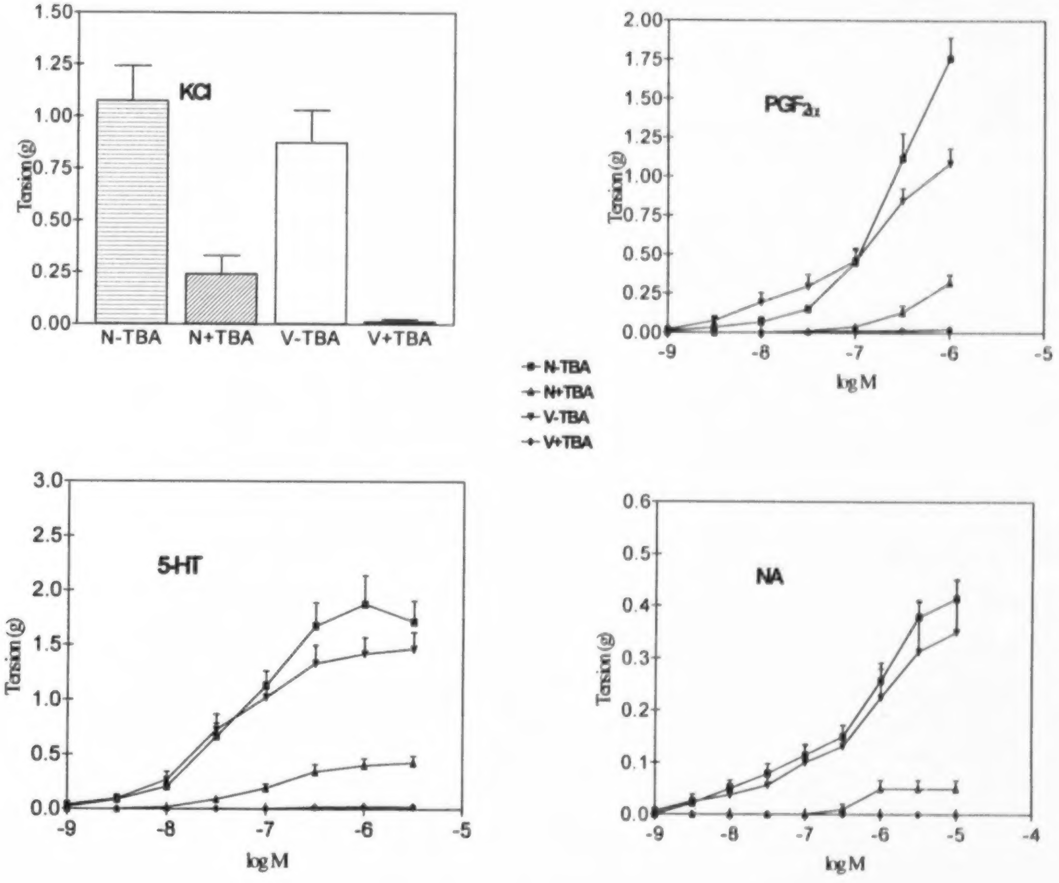
KCl
Tension (g)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
N-TBA
N+TBA
V-TBA
V+TBA
PGF2α
Tension (g)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-9
-8
-7
-6
-5
log M
N-TBA
N+TBA
V-TBA
V+TBA
5-HT
Tension (g)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-9
-8
-7
-6
-5
log M
NA
Tension (g)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-9
-8
-7
-6
-5
-4
log M

Figure 9

Figure 10. Effects of transluminal balloon angioplasty (TBA) in endothelium-dependent relaxation of normal vessels. Vasorelaxation studies using calcium ionophore (A_{23187}) and bradykinin (BK) for 3 groups of vessels: a) normal vessels without TBA (N-TBA); b) normal vessels with TBA (N+TBA); and c) vasospastic vessels without TBA (V-TBA). Percent relaxation was calculated using steady-state contraction at 10^{-6} M $PGF_{2\alpha}$ as 0% relaxation. N+TBA showed significant reduction or abolition of endothelium-dependent vasorelaxation in comparison to N-TBA or V-TBA ($p<0.05$). No significant difference was noted between N-TBA and V-TBA for both BK and A_{23187}.

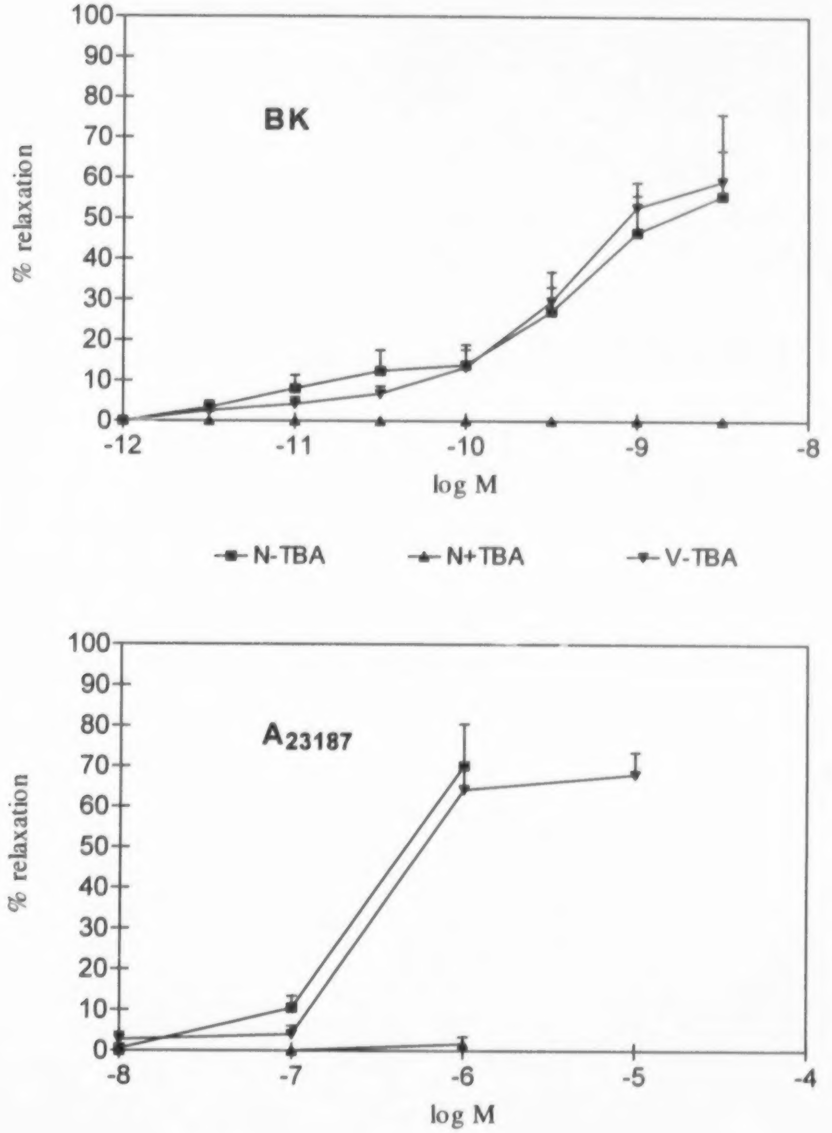
BK
% relaxation
100
90
80
70
60
50
40
30
20
10
0
-12
-11
-10
-9
-8
log M
N-TBA
N+TBA
V-TBA
A_{23187}
% relaxation
100
90
80
70
60
50
40
30
20
10
0
-8
-7
-6
-5
-4
log M

Figure 10

Figure 11.Scanning electron micrographs. End-on view of a normal (A) and vasospastic vessel (B). Compared to the normal vessel, the vasospastic vessel (B) shows smaller luminal diameter and thicker vessel wall.

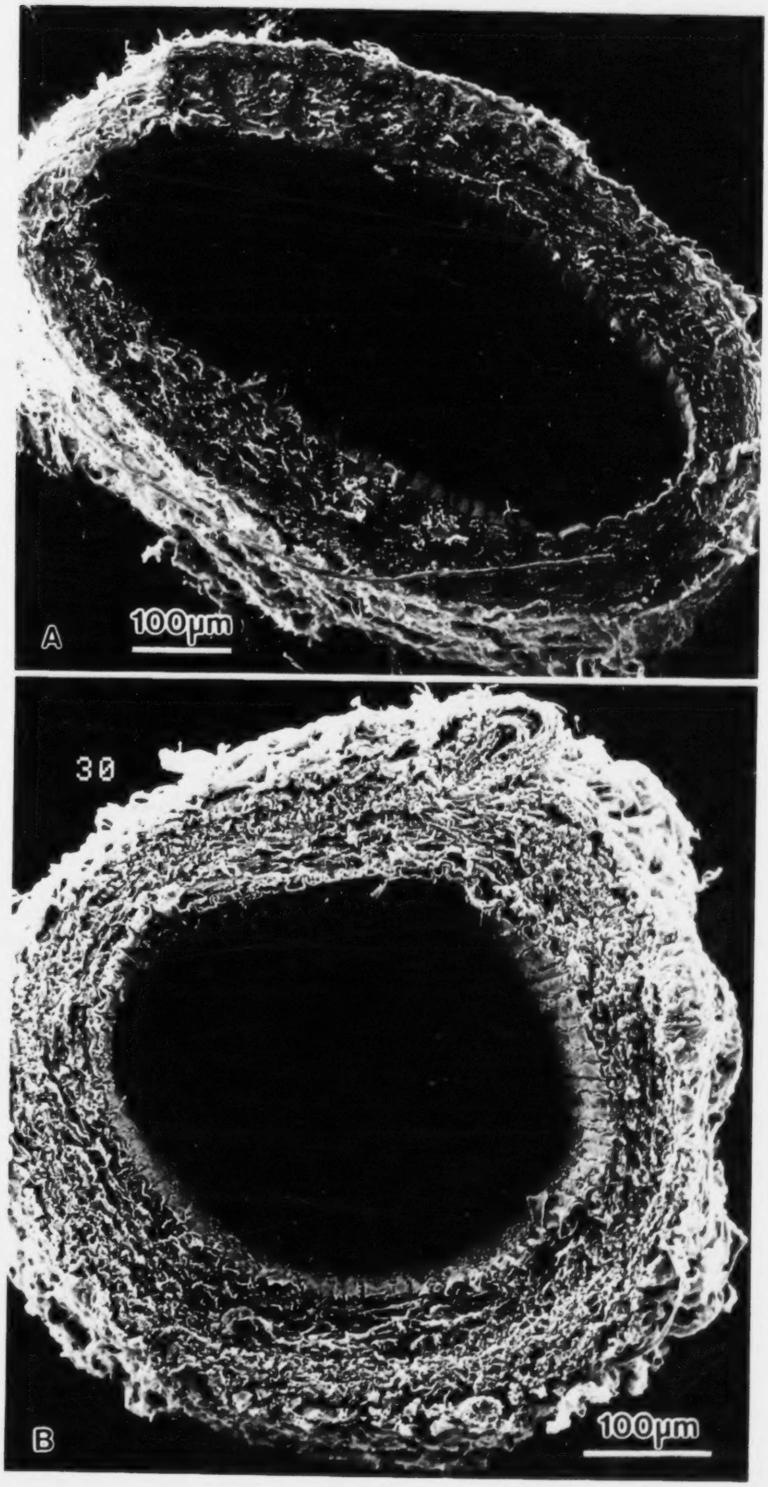
A
100µm
30
B
100µm

Figure 11.

Figure 12. Scanning electron micrographs. A: Smooth muscle layer was exposed after hydrochloric acid digestion. B: Extracellular matrix was exposed after bleach digestion, which eliminated cellular components such as smooth muscle cells.

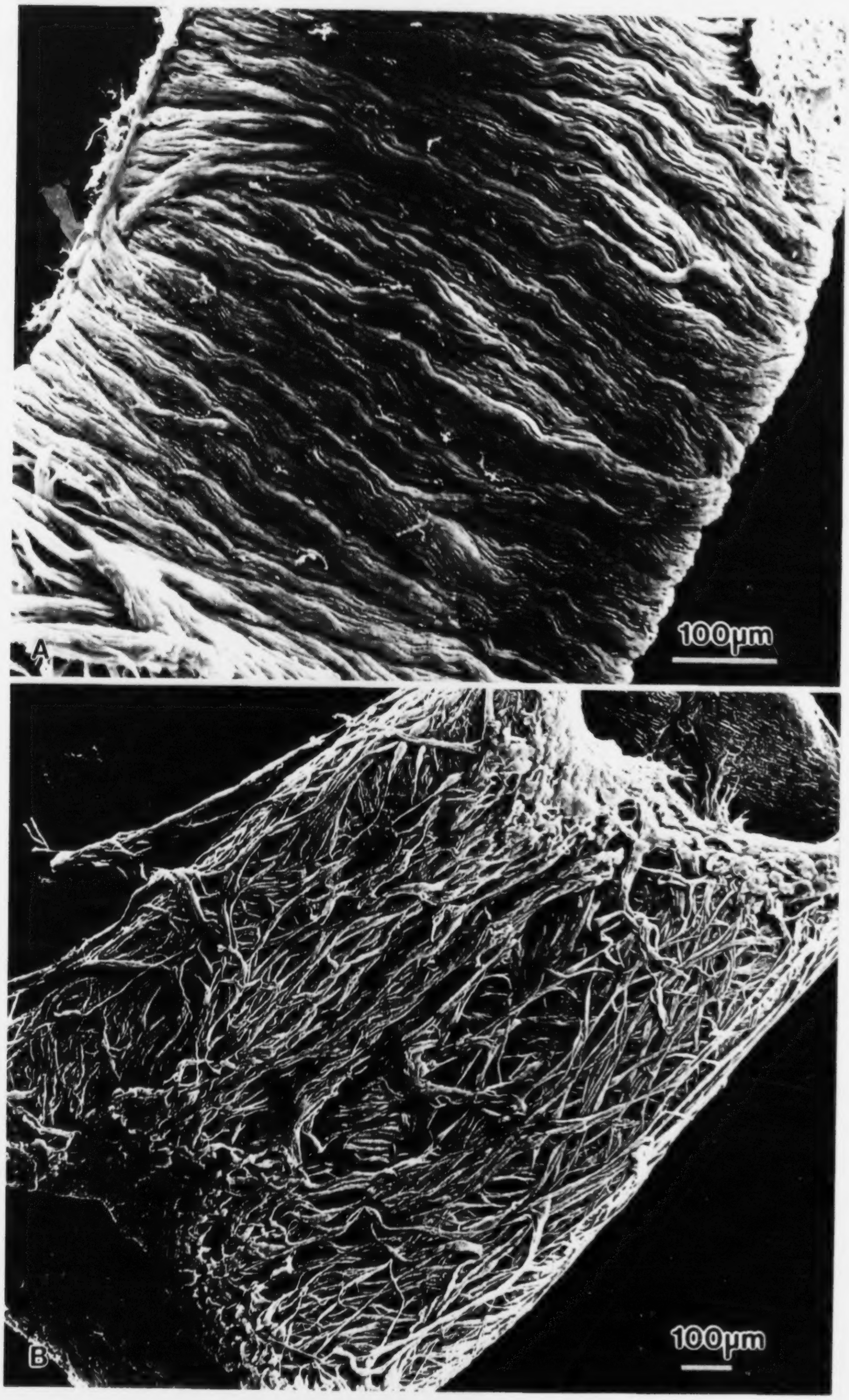
A
100µm
B
100µm

Figure 12.

Figure 13. Effects of transluminal balloon angioplasty (TBA) on smooth muscle cells and extracellular matrix of vasospastic vessels as seen on scanning electron microscopy. A: smooth muscle cells from a dilated vasospastic artery showing flattening of surface rippling; B: smooth muscle cells from a non-dilated vasospastic artery showing multiple surface ripplings (RP). C & C1: low- and high-power view, respectively, of the extracellular matrix from a dilated vasospastic vessel. D & D1: low- and high-power view, respectively, of the extracellular matrix from a nondilated vasospastic vessel (HOR= horizontal fibers; LGT= longitudinal fibers). No difference was noted between C and D, as well as C1 and D1. Particularly, no fiber breakage was noted in C1 and D1.

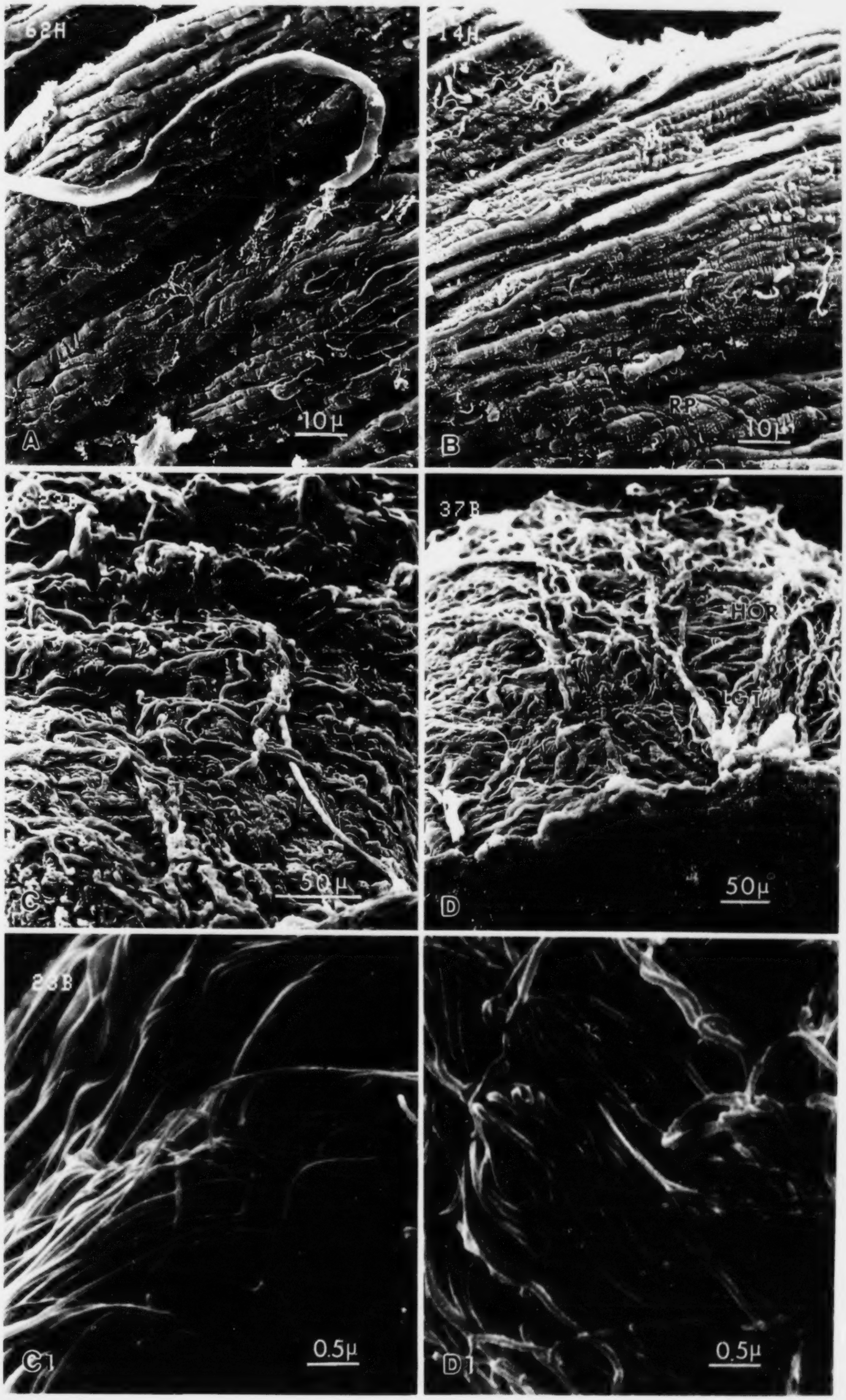
62H
14H
A
10μ
B
RP
10μ
37B
HOR
LGT
C
50μ
D
50μ
23B
C1
0.5μ
D1
0.5μ

Figure 13.

Figure 14. Effects of transluminal balloon angioplasty (TBA) on luminal diameter and endothelium of vasospastic vessels as seen on scanning electron microscopy. A: nondilated vasospastic vessel segment showing narrow vessel lumen; B: dilated vasospastic vessel segment from the same dog showing enlarged luminal diameter; C: intact but corrugated endothelium (EDT) of nondilated vasospastic vessel; D: flattened (FLT; arrow in inset) endothelium with patchy denudation (DND; arrow in inset) from a dilated vasospastic vessel; Inset D: High-power view of areas denoted DND and FLT.

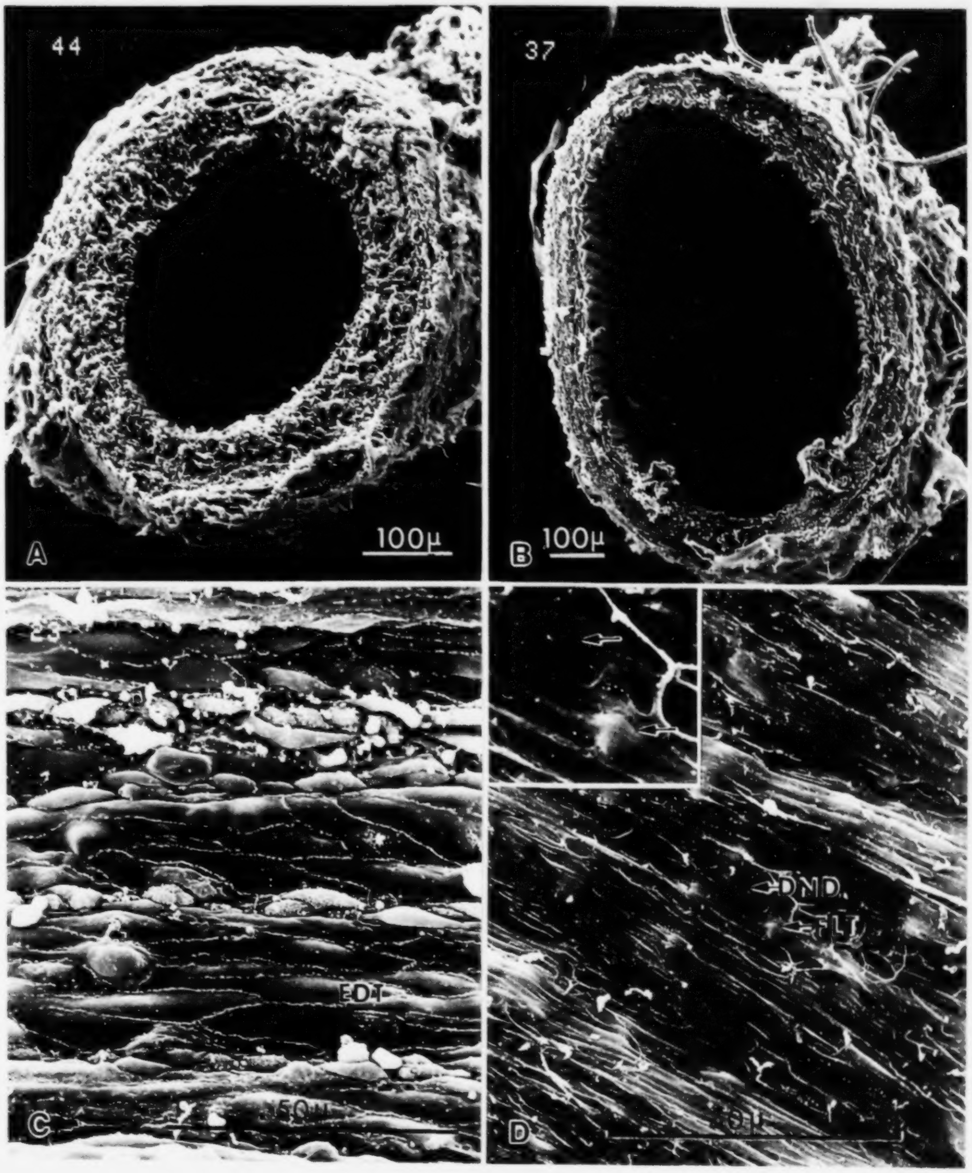
44
37
A
100μ
B
100μ
EDT
C
50μ
DND
FLT
D

Figure 14.

Figure 15. Effects of transluminal balloon angioplasty (TBA) in a normal vessel as seen in cross-section of the vessel wall with transmission electron microscopy. A: normal vessel without TBA showing intact endothelium (EDT), internal elastic lamina (IEL), smooth muscle cells (SMC), extracellular matrix (ECM), and the tunica adventitia (ADV); B: normal vessels treated with TBA showed endothelial flattening (EDT), straightening and thinning and occasional rupturing (*) of internal elastic lamina (IEL), mild crowding and straightening of smooth muscle cells (SMC). (ADV= tunica adventitia).

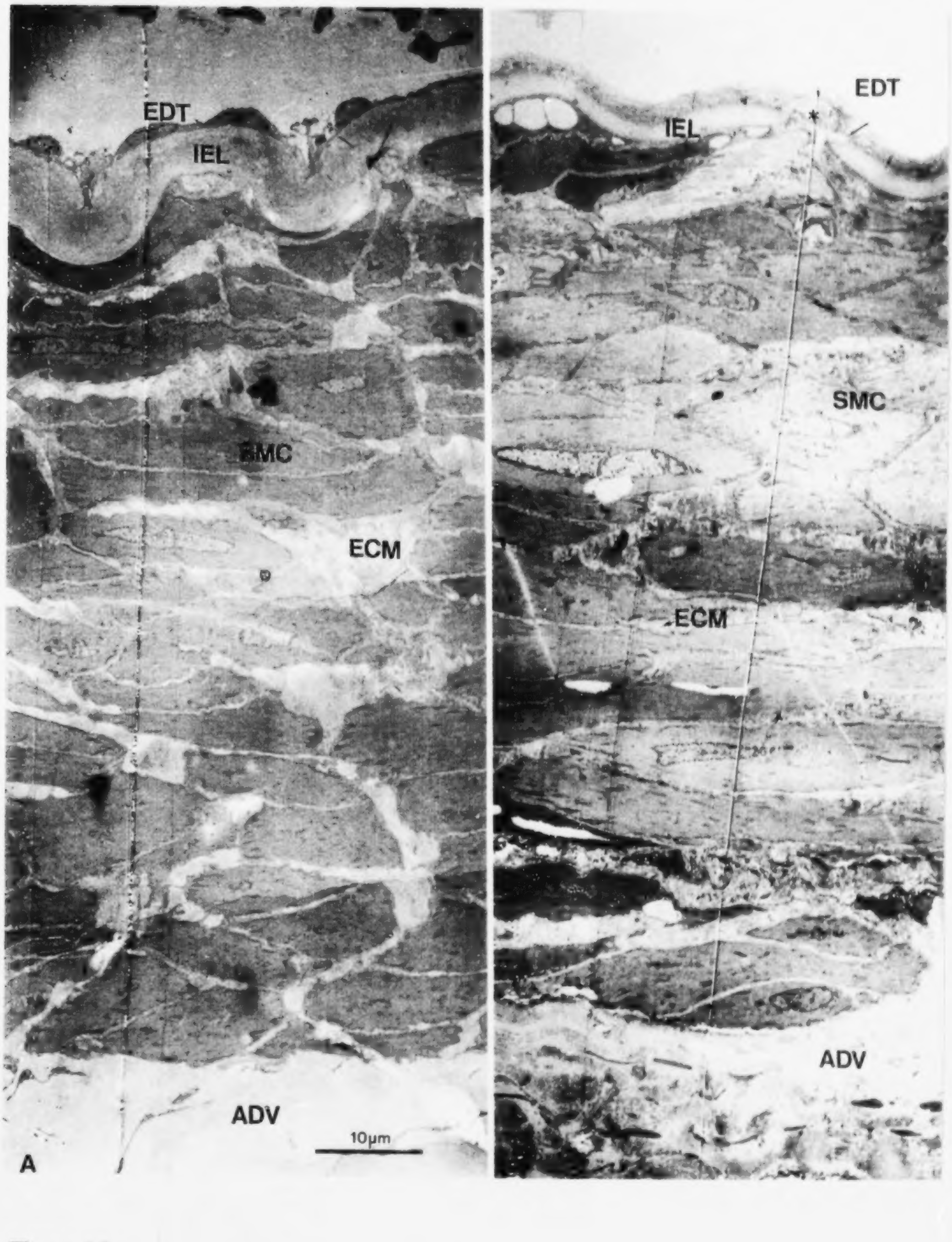
EDT
IEL
SMC
ECM
ADV
10μm
A
EDT
IEL
SMC
ECM
ADV

Figure 15.

Figure 16. Effects of transluminal balloon angioplasty (TBA) in a vasospastic vessel as seen in cross-section of the vessel wall with transmission electron microscopy. A: vessel segment without TBA showing endothelial cells (EDT), corrugated internal elastic lamina (IEL), smooth muscle cells (SMC) with surface rippling (RP), extracellular matrix (ECM), and the tunica adventitia (ADV); B: vessel segment dilated with TBA showing flattening of endothelium (EDT), straightening and thinning of internal elastic lamina (IEL), stretching and straightening smooth muscle cells with flattening of surface rippling (SB), and thinning of the tunica media.

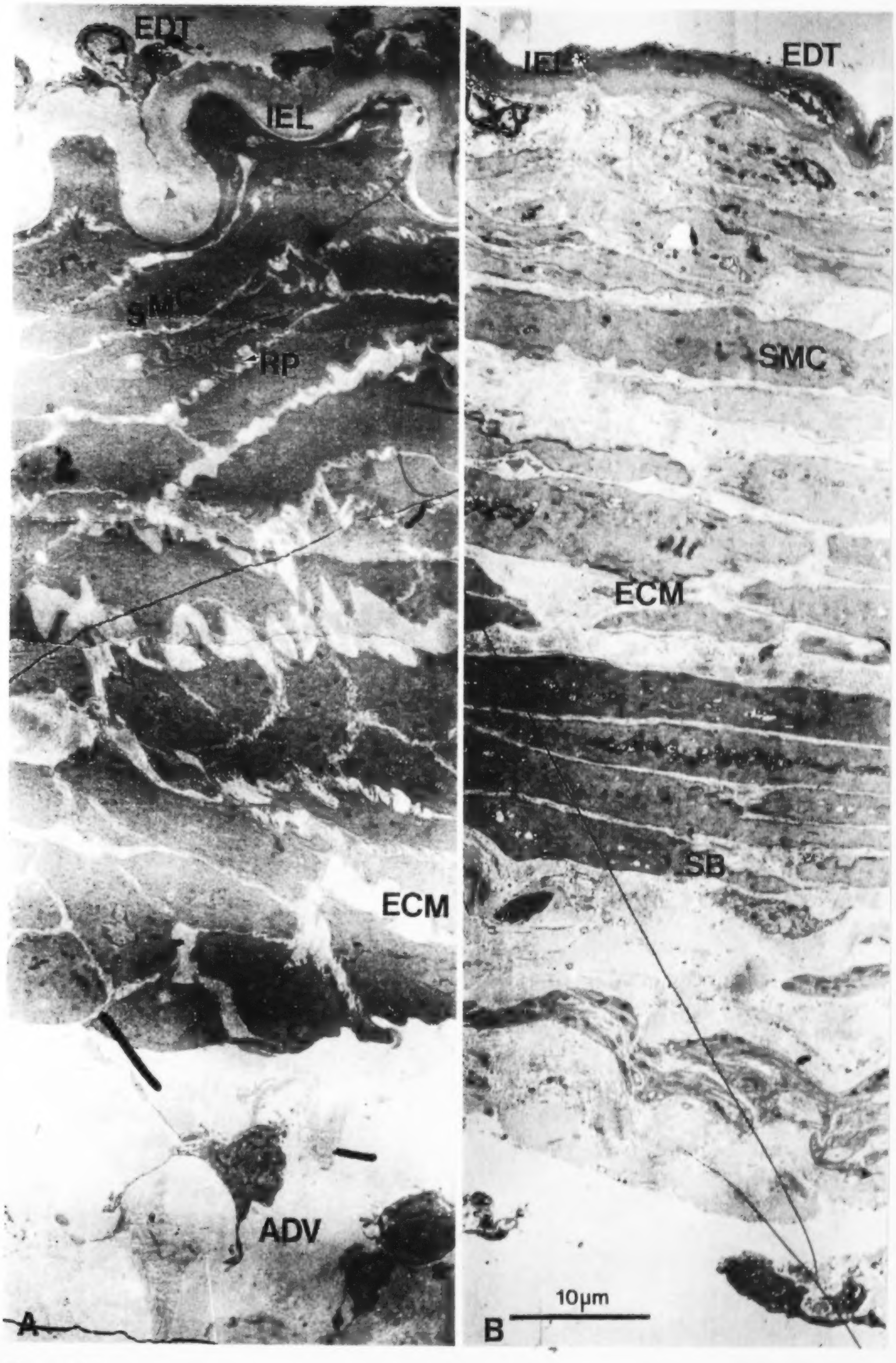
EDT
IEL
SMC
RP
ECM
ADV
A
IEL
EDT
SMC
ECM
SB
10µm
B

Figure 16.

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